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# PLANTERS' RECORD

VOL. XL

A quarterly paper devoted to the sugar interests of Hawaii,  
and issued by the Experiment Station for circulation among  
the plantations of the Hawaiian Sugar Planters' Association.

JANUARY

TO

DECEMBER

# THE HAWAIIAN PLANTERS' RECORD

VOL. XL

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3-7-39

ORGAN OF THE EXPERIMENT STATION OF THE  
HAWAIIAN SUGAR PLANTERS' ASSOCIATION

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## TABLE OF CONTENTS

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	Page
Diagnosing the Status of Available Phosphoric Acid and Potash in Sugar Cane Soils.....	3
Some Interesting Cane-Tonnage, Purity, Soil-Analyses, Juice-Analyses Relationships .....	11
Some Aspects of the Internal Water Economy of the Sugar Cane Plant .....	21
The Effect of Nitrogen on Cane Yield and Juice Quality .....	35
Biological Control of the Sugar Cane Leafhopper in Hawaii .....	57
The Day-Degree in Mauritius.....	103
Sugar Prices .....	104
A Suggested Scheme of Irrigation Control Using the Day-Degree System .....	109
Studies in Experimental Technique.....	113
POJ 2878 Cane in the Factory.....	117
Utilization of Molasses .....	121
The Third International Congress of Soil Science.....	127
Notes on <i>Pythium</i> Root Rot—VIII.....	137
Cane Growth Studies .....	143
Rat Control Investigations at The Lihue Plantation Company, Ltd. ....	157
Yams for Hawaiian Gardens.....	171
Primary and Total Combustion Volume in Factory Boilers .....	183
Mill Roller Openings.....	183
Sugar Prices .....	185
Soil and Plant Material Analyses by Rapid Chemical Methods .....	189
Sugar Prices .....	300
A Giant Macadamia Nut.....	303
A Preliminary Report on an Entomological Survey of Guam .....	307
Comparative Hardness of Tasseled Versus Untasseled Canes .....	315
Soil Reaction and Total Acidity.....	319
The Fluctuations of Sugars in the Leaf Sheaths of the Sugar Cane Plant During the Day and the Night...	329
Further Notes on Water and Cane Ripening.....	355
Sugar Prices .....	382

ILLUSTRATIONS APPEARING ON THE COVERS OF  
VOLUME XL

FIRST QUARTER



FLOWER OF THE BALSA TREE

Balsa, the lightest wood known, should not be overlooked as a possible crop for idle lands in Hawaii.



SECOND QUARTER



A few of the large stools of sugar cane exhibited at the Kauai Fair.

The stool directly behind the two girls at the left took first prize in its class and was also named Grand Champion. It is a stool of 30 2417 and was entered by Kekaha Sugar Company, Ltd.

THIRD QUARTER



The Experiment Station, U.S.P.A. "Potash Rotator."

#### FOURTH QUARTER



A fruiting branch of the Giant Macadamia Nut of North Queensland, exhibited by G. Windred, Chief Entomologist of the Colonial Sugar Refining Company. Efforts towards introduction and establishment in Hawaii are now underway.





3-7-39

# THE HAWAIIAN PLANTERS' RECORD

Vol. XL

FIRST QUARTER, 1936

No. 1

*A quarterly paper devoted to the sugar interests of Hawaii and issued by the Experiment Station for circulation among the plantations of the Hawaiian Sugar Planters' Association.*

## **In This Issue:**

### *Diagnosing the Status of Available Phosphoric Acid and Potash in Sugar Cane Soils:*

Definite soil fertility zones, showing differences in the available supply of phosphate and of potash as determined by the rapid chemical methods of analyses of soil and crusher juice samples, have been located in fields of Pioneer Mill Company, Ltd., that were harvested in 1935. A good relationship was found between the cane yield and the available status of each nutrient in those areas where another limiting growth factor was not dominant. Hence the possibilities for the utilization of the analytical results secured by the rapid chemical tests to develop a sound economic plan of differential fertilization is clearly demonstrated.

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### *Cane-Tonnage, Purity, Soil-Analyses, Juice-Analyses Relationships:*

In the analytical data secured by the Pioneer Mill Company, Ltd., during 1935 we have found some very interesting relationships between (a) cane yields and cane quality, (b) cane yields and available phosphate and potash, (c) available phosphate and potash supply in the soil with the soil reaction, (d) available mineral nutrients in the soil and their corresponding uptake by the cane plant as indicated by amounts found in the crusher juice, and (e) phosphate and potash in crusher juice with the corresponding purity of such juice. These relations are apparent in the summarized data which are offered thereafter.

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### *Some Aspects of the Internal Water Economy of the Sugar Cane Plant:*

The use of the hand refractometer in a field of H 109 cane at Pioneer Mill Company, Ltd., has provided some evidence that the Brix of that variety suffers a diurnal variation, being relatively high in the late afternoon and low in the morning. This variation is tentatively attributed to variations in the moisture content of the cane, rather than to variations in the actual sugar content.

Although the results were substantiated at the Waipio substation, no convincing verification was secured at the Hilo Variety Station under the environmental and varietal conditions at that place.

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*The Effect of Nitrogen on Cane Yield and Juice Quality:*

This paper gives a rather non-technical account of what happens to the rate of growth, yield, juice quality and chemical composition of H 109 cane when grown with low, medium or very heavy nitrogen fertilization. The experiment is one of a series of studies which has for its ultimate purpose the evaluation of all the factors that concern cane quality.

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*Biological Control of the Sugar Cane Leafhopper:*

A historical account is given of the early appearance and spread of the leafhopper throughout the sugar cane districts of Hawaii and of the search for parasites in other countries and their introduction. An account of each parasite and its value as an enemy of the leafhopper is also given. *Cyrtorhinus mundulus* from Fiji and Australia, a bug which sucked leafhopper eggs, was found to be of greatest importance. There is also an account of many native insects and spiders which assisted in bringing about the ultimate control of the leafhopper.

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*The Day-Degree in Mauritius:*

A brief comment is given on the use of the day-degree in Mauritius.

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## Diagnosing the Status of Available Phosphoric Acid and Potash in Sugar Cane Soils

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By R. J. BORDEN

Making use of the rapid chemical methods (1) of analyzing soils and crusher juices for phosphoric acid and potash, and of the official methods of the Association of Hawaiian Sugar Technologists (2) for determining Brix and polarization and calculating purity of crusher juices, the field and technical staff of Pioneer Mill Company, Ltd.,\* have cooperated in a very thorough and extensive study to determine the status of available phosphoric acid and potash in all fields that were harvested in 1935. The plan and procedure for this study have been presented by Taylor (3) but are given here again in a brief form so that the results reported hereafter may be intelligently studied.

In the procedure as used by Taylor, an attempt is made to choose a field sampling station to represent every five acres from which cane is being harvested. Thus in a 50-acre field, some 10 sampling stations would be selected and distributed over the total area in such a way as to pretty generally "sample" the field. The area of each of these sampling stations is that of 4 or 5 adjacent watercourses occupying from 0.3 to 0.5 acre. These stations are selected and "flagged out" (with a yellow flag at each of the 4 corners) immediately after the cane has been cut and the track line installed nearby so that all cane from the sampling station can be loaded upon cars in an unbroken string. The ticket boy and harvesting luna supervise the loading and mark the cars with an identifying cane ticket and level ditch number so that all concerned are well advised. At the mill, the cane is weighed and a running crusher juice sample of all cane cut from each station is taken for the various juice analyses that are desired. After the cane is loaded, a soil sample is taken from the area of the sampling station. A definite procedure is used to collect this soil sample: in each watercourse of the selected area, starting at a point 10 feet inside the watercourse in the fifth line and similarly in every fifth line therefrom, the top 4 or 5 inches of soil are scraped away from the lower portion of the mauka bank and about 2 handfuls of soil are then dug out and placed in a bucket. When some 20 to 25 of these unit samples have thus been taken up, they are thoroughly mixed together and about 1½ pounds of this well-mixed soil is put into a paper bag and brought to the soil laboratory for the required soil analysis. Later on, before the corner flags have been removed, the area of each sampling station in the field is measured so that its cane weight in tons per acre may be calculated from the weight of cane that was obtained. Sufficient information is taken at this time so that each sampling station can be relocated for future soil sampling in the field.

As a result of this procedure, which has called for the active cooperation of the cutters and loaders, the harvesting and transportation bosses, the yard and scale

\* We are greatly indebted to the officials of the Pioneer Mill Company, Ltd., who have made their data available to us for the study which forms the basis of this presentation.



men, the technical staffs of the mill chemist, the civil engineer, and the agriculturist, we have a large body of analytical data which is truly correlative, since cane weights, cane juices, and soil analyses have all been secured from the same identical field areas.

Sampling errors are undoubtedly represented in the data but because there are an adequate number of samples, the influence of such errors is perhaps not such as to seriously affect the averages or to cover up the trends that are apparent. Discrepancies between soil and juice analyses have also occurred and it is quite likely that a verification of the status of the mineral nutrients in the soil, by resampling the specific sampling stations where disagreement is observed, will improve the reliability of the data.

We believe, however, that the analytical data that have been secured show some very definite results which we shall now attempt to point out and to briefly discuss for the various sections of the plantation and for a few of the larger individual fields that are represented in the data.

#### STUDY No. 1

##### Relation of Available Phosphate to Cane and Sugar Yields in Field C-2.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	P <sub>2</sub> O <sub>5</sub> in the Juice	Tons Cane per Acre	Tons Sugar per Acre
23	Doubtful-Low	.035 per cent	66.6	8.28
16	High	.040 per cent	79.1	9.98
—				
39				

The supply of available potash in this field is all medium or high.

Comment: The better cane and sugar yields came from areas that had a "high" supply of phosphate. The status of a few smaller areas in this field needs verification, but in general the area divides itself into the upper half which is well supplied with phosphate, and the lower half which may be deficient. If, as is thought to be the case, this high phosphate supply has resulted from applications of filter cake that were made to this normally low-phosphate soil several years ago, then an inspection of the field-fertility map (Fig. 1) will clearly indicate where future applications of filter cake might be made to best advantage in this field.

#### STUDY No. 2

##### Relation Between Phosphate and Potash in the Soil and Juice, and the Cane Yields in Field B-6.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	P <sub>2</sub> O <sub>5</sub> in the Juice	Cane Yields: Tons per Acre
16	Doubtful	.036 per cent	73.6
12	Medium-High	.054 per cent	85.8
—			
28			
No. of Samples Averaged	K <sub>2</sub> O in the Soil	K <sub>2</sub> O in the Juice	Cane Yields: Tons per Acre
11	Doubtful-Low	.134 per cent	73.3
17	Medium	.233 per cent	82.4
—			
28			

Comment: Both the potash and the phosphate differences in the soil of this field appear to be related to the cane yields. The juice data substantiate the soil data. The different zones of fertility in this field are quite clearly defined and a differential fertilization would be practicable.

# PIONEER MILL CO. FIELD C-2 1935 CROP 181.4 ACRES

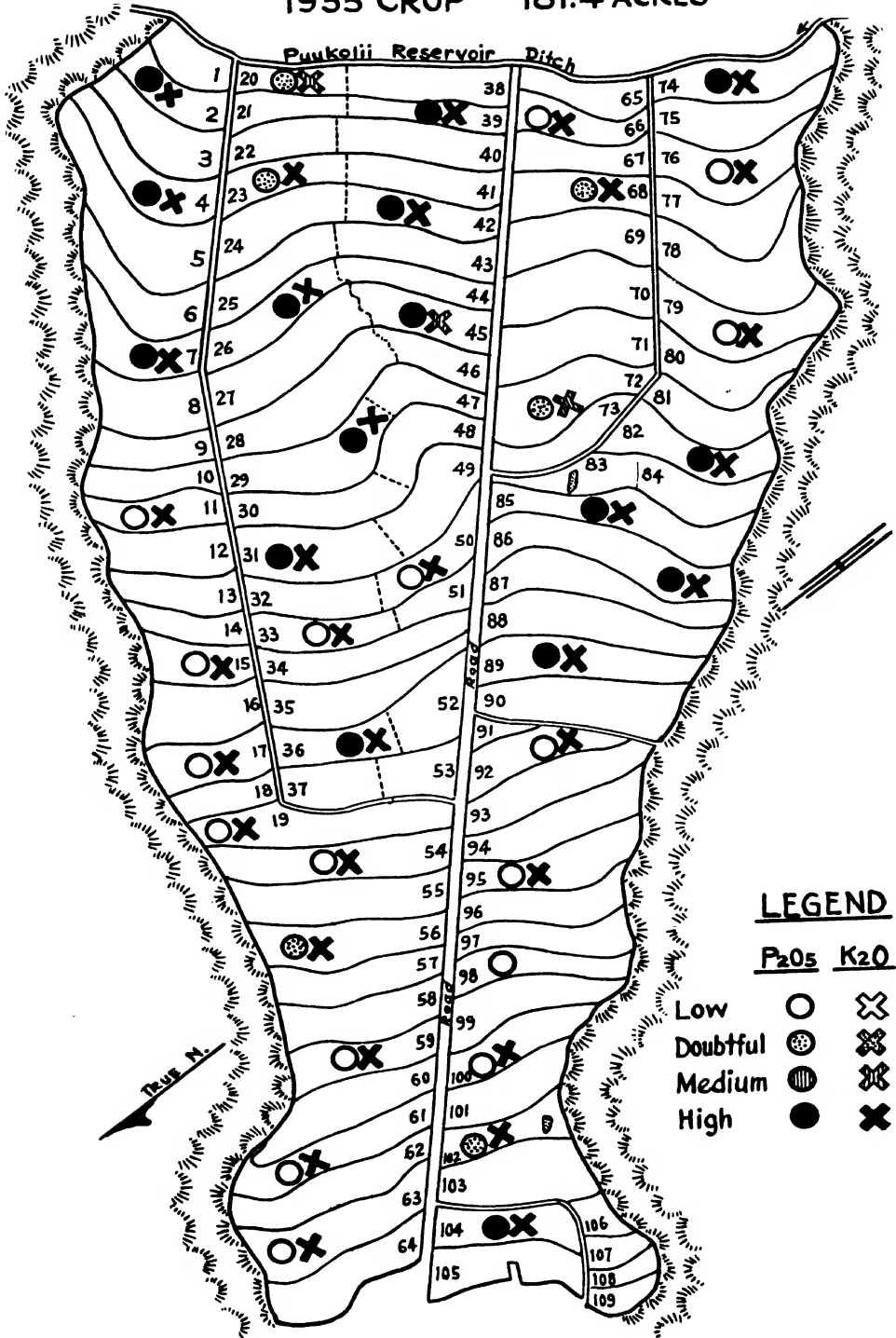


Fig. 1

## STUDY No. 3

## Relation Between Phosphate and Potash in the Soil and Juice, and the Cane Yields in Field 32.

No. of Samples Averaged		P <sub>2</sub> O <sub>5</sub> in the Soil	P <sub>2</sub> O <sub>5</sub> in the Juice	Cane Yields: Tons per Acre
Soil	Juice			
13	14	Doubtful-Low	.020 per cent	83.7
17	19	Medium-High	.026 per cent	86.6
—	—			
30	33			

No. of Samples Averaged		K <sub>2</sub> O in the Soil	K <sub>2</sub> O in the Juice	Cane Yields: Tons per Acre
Soil	Juice			
8	9	Doubtful-Low	.114 per cent	78.6
23	24	Medium-High	.260 per cent	88.2
—	—			
31	33			

Comment: Here we note a possibly greater influence of the potash than of the phosphate supply on cane yields. The zones of the higher and of the lower mineral fertility in this field are quite distinct.

## STUDY No. 4

## Relation of Potash in the Soil and Juice to Cane Yields in Field 33.

No. of Samples Averaged		K <sub>2</sub> O in the Soil	K <sub>2</sub> O in the Juice	Cane Yields: Tons per Acre
11		Doubtful-Low	.143 per cent	71.8
19		Medium	.165 per cent	76.2
—				
30				

Comment: A relationship between the available potash and cane yields is indicated here, in spite of the fact that the phosphate supply is low for all samples in this field.

## STUDY No. 5

## Relation Between Phosphate and Potash in the Soil and Juice, and the Cane Yields in Field 34.

No. of Samples Averaged		P <sub>2</sub> O <sub>5</sub> in the Soil	P <sub>2</sub> O <sub>5</sub> in the Juice	Cane Yields: Tons per Acre
Soil	Juice			
33	34	Doubtful-Low	.026 per cent	67.9
13	13	Medium-High	.032 per cent	70.1
—	—			
46	47			

No. of Samples Averaged		K <sub>2</sub> O in the Soil	K <sub>2</sub> O in the Juice	Cane Yields: Tons per Acre
Soil	Juice			
35	35	Doubtful-Low	.160 per cent	68.6
11	10	Medium	.179 per cent	69.7
—	—			
46	45			

Comment: The influence of potash and phosphate on these yields is not very great. Both plant foods appear generally low.

## STUDY No. 6

Relation Between Phosphate in the Soil and the Cane Yields in the "C" Fields.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	Tons Cane per Acre
23	Low-Doubtful	66.2
42	Medium-High	83.3
—		
65		

Comment: A very definite relation between cane yields and the supply of available phosphate is shown in the "C" fields. All samples were high in potash.

## STUDY No. 7

Relation Between Phosphate and Potash in the Soil and the Cane Yields in the "B" Fields.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	Tons Cane per Acre
75	Low-Doubtful	75.9
34	Medium-High	77.7
—		
109		

No. of Samples Averaged	K <sub>2</sub> O in the Soil	Tons Cane per Acre
36	Low-Doubtful	68.0
73	Medium-High	80.9
—		
109		

Comment: Potash apparently dominates the effect on cane yields in the "B" fields.

## STUDY No. 8

Relation Between Phosphate in the Soil and Cane Yields in the "G" Fields.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	Tons Cane per Acre
10	Low-Doubtful	72.4
20	Medium-High	76.8
—		
30		

Comment: In the "G" fields, the deficiency of available potash which is mostly low to doubtful, has somewhat limited the full influence of the phosphate on the cane yields.

## STUDY No. 9

Relation Between Phosphate and Potash in the Soil and Cane Yields in the "H" Fields.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	Tons Cane per Acre
29	Low-Doubtful	78.4
36	Medium-High	77.4
—		
65		
No. of Samples Averaged	K <sub>2</sub> O in the Soil	Tons Cane per Acre
33	Low-Doubtful	79.2
32	Medium-High	76.8
—		
65		

Comment: No effect upon cane yields is indicated by the amounts of available phosphate and potash found in the "H" fields. This suggests that (a) other growth factors than these mineral nutrients have a more effective influence on cane yields, and (b) an adjustment of fertilizer applications may not be expected to raise the average cane yields here until other limiting factors are corrected.

## STUDY No. 10

## Relation Between Phosphate in the Soil and Cane Yields in the "F" Fields.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	Tons Cane per Acre
44	Low-Doubtful	83.5
7	Medium-High	75.0
—		
51		

Comment: An effect of the available phosphate upon the cane yields is not apparent in the "F" fields where potash is apparently deficient. (Samples all show low to doubtful potash.)

## STUDY No. 11

## Relation Between Phosphate and Potash in the Soil with Cane Yields in the "MA" Fields.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	Tons Cane per Acre
7	Low-Doubtful	65.2
14	Medium-High	75.2
—		
21		

No. of Samples Averaged	K <sub>2</sub> O in the Soil	Tons Cane per Acre
6	Low-Doubtful	66.1
15	Medium-High	74.2
—		
21		

Comment: The higher cane yields in the "MA" fields are quite definitely related to the available supply of both phosphate and potash.

## STUDY No. 12

## Relation of Phosphate and Potash in the Soil to Cane Yields in the Honokowai Fields.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	Tons Cane per Acre
164	Low-Doubtful	75.0
44	Medium-High	80.3
—		
208		

No. of Samples Averaged	K <sub>2</sub> O in the Soil	Tons Cane per Acre
60	Low-Doubtful	72.2
149	Medium-High	77.7
—		
209		

Comment: A small, but yet rather definite, influence of both phosphate and potash upon cane yields is noted in the Honokowai fields.

## STUDY No. 13

Relation of Phosphate and Potash in the Soil to Cane Yields in the "LB" Fields.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	Tons Cane per Acre
26	Low-Doubtful	68.1
26	Medium-High	66.1
—		
52		
No. of Samples Averaged	K <sub>2</sub> O in the Soil	Tons Cane per Acre
42	Low-Doubtful	62.9
10	Medium-High	74.9
—		
52		

Comment: In the "LB" fields, potash apparently exerts a greater effect on cane yields than phosphate.

## STUDY No. 14

Relation of Potash in the Soil to Cane Yields in the "LC" Fields.

No. of Samples Averaged	K <sub>2</sub> O in the Soil	Tons Cane per Acre
8	Low-Doubtful	64.5
40	Medium-High	71.5
—		
48		

Comment: In the "LC" fields where the supply of phosphate was uniformly high, available soil potash is apparently a cane yield-effect factor.

## STUDY No. 15

Relation of Phosphate and Potash in the Soil to the Cane Yields Secured in the Olowalu Section.

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in the Soil	Tons Cane per Acre
13	Low-Doubtful	68.7
68	High	75.0
—		
81		
No. of Samples Averaged	K <sub>2</sub> O in the Soil	Tons Cane per Acre
31	Low-Doubtful	72.9
50	Medium-High	73.8
—		
81		

Comment: The influence of available soil phosphate on cane yields appears to be a greater factor than the influence of potash in the fields at Olowalu.

### Conclusion:

The data studied are reliable and adequate; hence the conclusions that are drawn can be sound if they have been properly deduced.

Rather definite zones with mineral fertility differences—i.e., of high and medium phosphate, of doubtful and low phosphate, of high and medium potash, and of doubtful and low potash—have been located within many of the fields. The fertility status

in these zones is in most instances directly related to the sugar cane tonnage that was harvested from them.

Wherever this direct relationship between cane tonnage and available mineral nutrients is found, it suggests that a plan of differential fertilization can be employed to (a) build up the available nutrient supply in the "low-doubtful" areas, and thereby raise the average cane yield for the field, and (b) save money that would ordinarily be expended for fertilizer for application on the "medium-high" areas, since our previous studies have definitely indicated that a yield response to either phosphate or potash fertilizer is not likely to be secured where the supply of these fertilizers in our cane soils is indicated as "high" by our rapid chemical methods of analyses. However, when the relationship between the cane yields and the supply of available nutrients is not found, it may be questionable whether we can raise the average cane yield for such a field, by any adjustment of the fertilization, until the more dominant limiting growth factor has been ascertained and corrected.

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- (1) Hance, F. E., 1935. Report of the Chemistry department, Report of the Committee in Charge of the Experiment Station, H. S. P. A., pp. 76-88.
- (2) Chemical Control for Cane Sugar Factories, 1931. Ass'n of Haw'n Sugar Tech., 143 pp.
- (3) Taylor, H. J. W., 1935. A procedure for sampling cane juice and soil as used at Pioneer Mill Company, Ltd., The Hawaiian Planters' Record, Vol. XXXIX, pp. 109-112.

#### *Addenda:*

In using the rapid chemical methods for determining the available status of phosphoric acid and potash in soils, four qualitative groupings or classes are used, e. g., "High," "Medium," "Doubtful" and "Low." For those who would prefer to use "parts per million" or "pounds per acre-foot of soil," the following table is offered.

CLASS	PHOSPHORIC ACID (1)		POTASH (2)	
	P. P. M.	Pounds per Acre-foot	P. P. M.	Pounds per Acre-foot
Low.....	Less than 8	Less than 20	Less than 70	Less than 175
Doubtful.....	8 to 15	20 to 38	70 to 140	175 to 350
Medium.....	15 to 40	38 to 100	140 to 280	350 to 700
High.....	More than 40	More than 100	More than 280	More than 700

(1) Soluble in a dilute solution of hydrochloric acid during a period of one-half minute.

(2) Soluble in a dilute acid solution of sodium acetate during a period of one-half minute.

## Some Interesting Cane-Tonnage, Purity, Soil-Analyses, Juice-Analyses Relationships

By R. J. BORDEN

The analytical data that were gathered by the technical staff of the Pioneer Mill Company, Ltd., from soil and crusher juice samples collected in connection with their efforts to determine the status of available phosphate and potash in their sugar cane lands, have afforded an excellent opportunity to determine several relationships that exist between the various analyses that were made. We are privileged\* to offer herewith a summary of several studies that we have made from these data.

In presenting these studies from this analytical work, we have assumed various arithmetic groupings or various classes for one of the factors concerned, and thereafter have averaged the related figures for the other factor. It appeared impractical to add an indefinite number of regularly spaced arithmetic groupings to accommodate some very few cases; hence, our extreme groupings, which may be indicated as "Below ....., " or "Above ....., " are apt to contain the more grossly erratic figures which often result from sampling and analytical errors, and the averages concerned with these extreme groupings must be interpreted rather carefully, if they are used at all.

The averages are presented without their probable errors at this time, since it is assumed that we are interested in the trends and general correlations, rather than in arriving at the significance attached to the specific amounts of difference observed. A short comment concerned with our interpretation of the summarized data is offered with each individual study.

We believe that these data have shown some rather definite results, which we suggest are as follows:

- (1) That the higher cane yields had a poorer quality ratio than the average yields, but that this fact in itself may be of little economic importance.
- (2) That a higher content of both available phosphate and available potash was found in the neutral-to-slightly-alkaline soils than in the acid soils.
- (3) That there was a direct relationship between the available phosphate in the soil and the percentage of phosphate found in the crusher juice of cane grown on such soil; similarly, that this direct relationship occurred between available soil potash and the crusher juice potash.
- (4) That *both* phosphate and potash in the crusher juice showed an inverse relationship to the purity of this juice; this same relationship also holds for quality ratio.
- (5) That sugar cane yields were, to a large degree, directly related to the supply of available phosphate, when potash was not a limiting factor.
- (6) That sugar cane yields were also directly related to the supply of available potash, when phosphate was not a limiting growth factor.

Hence, without further discussion, we offer these summarized data for six studies.

\* By permission of the Pioneer Mill Company, Ltd.



## STUDY No. 1

## Relation of Cane Yields to Quality Ratio.

No. of Samples Averaged	Tonnage Class	Tonnage Group	Q.R.	Calculated Probable Sugar Yield
11	35 - 40.4		7.3	5.1
17	40.5 - 45.4		7.7	5.5
19	45.5 - 50.4		7.8	6.1
40	50.5 - 55.4		7.7	6.8
63 (150)	55.5 - 60.4	(under 60 TCA)	7.7 (Avg. 7.6)	7.5 (Avg. 6.2)
<hr/>				
85	60.5 - 65.4		7.6	8.2
99	65.5 - 70.4		7.7	8.7
115	70.5 - 75.4		7.5	9.7
110 (409)	75.5 - 80.4	(60-80 TCA)	7.6 (Avg. 7.6)	10.2 (Avg. 9.2)
<hr/>				
95	80.5 - 85.4		7.9	10.4
54	85.5 - 90.4		7.7	11.4
47	90.5 - 95.4		8.0	11.5
37 (233)	95.5 - 100.4	(80-100 TCA)	7.9 (Avg. 7.9)	12.3 (Avg. 11.4)
<hr/>				
16	100.5 - 105.4		8.2	12.5
14	105.5 - 110.4		8.0	13.4
14	110.5 - 115.4		8.3	13.6
9	115.5 - 120.4		9.2	12.8
15 (68)	Above 120.4	(over 100 TCA)	8.2 (Avg. 8.4)	14.9 (Avg. 13.4)

Total 860

Comment: The higher cane yields have generally resulted in a poorer quality ratio. From the calculated probable sugar yields, however, it would seem doubtful that this relationship is a factor that has adversely affected the economics of the sugar production, since the indicated larger sugar tonnages that are associated with the larger cane yields would have more than sufficient value to pay for the harvesting and milling costs on the extra cane tonnage that would be handled.

## STUDY No. 2

## Relation of Phosphate and Potash in the Soil to the pH of the Soil.

No. of Samples Averaged	Soil P <sub>2</sub> O <sub>5</sub> Group	pH of Soil
314	Low	6.4
176	Doubtful	6.4
99	Medium	6.6
343	High	7.0
<hr/>		
Total 932		

No. of Samples Averaged	Soil K <sub>2</sub> O Group	pH of Soil
205	Low	6.3
165	Doubtful	6.5
414	Medium	6.7
148	High	7.0
<hr/>		
Total 932		

Comment: Apparently there is a somewhat greater availability of phosphate and potash in neutral than in slightly acid soils.

## STUDY No. 3

Relations of (1) phosphate in the soil to phosphate in the crusher juice, and  
(2) potash in the soil to potash in the juice.

No. of Samples		
Averaged	P <sub>2</sub> O <sub>5</sub> in Soil	Per Cent P <sub>2</sub> O <sub>5</sub> in Juice
290	Low	.025
157	Doubtful	.029
99	Medium	.033
337	High	.042
<hr/>		
Total	883	

No. of Samples		
Averaged	K <sub>2</sub> O in Soil	Per Cent K <sub>2</sub> O in Juice
198	Low	.155
161	Doubtful	.179
385	Medium	.253
147	High	.320
<hr/>		
Total	891	

Comment: A definite positive relationship is shown between both phosphate and potash in the soil, and phosphate and potash in the juice.

On the basis of data obtained in this study, we may make a suggestion with regard to a tentative group classification for the various amounts of phosphate and potash that were found in the crusher juices. For the time being and in lieu of better criteria, we suggest that the following percentage values from crusher juices be used for the group classifications indicated. (This classification is for H 109 cane grown at Pioneer Mill Company, Ltd., and may not be reliable for other cane varieties or for H 109 grown under different conditions.)

Group Classification (To designate status of available nutrients)	Per Cent P <sub>2</sub> O <sub>5</sub> in the Crusher Juice	Per Cent K <sub>2</sub> O in the Crusher Juice
Low	.025	.15
Doubtful	.030	.20
Medium	.035	.25
High	.045	.35

It is thought that it will not be necessary to adjust these percentages for differences in purity, since the two following progressive relations are thought to be effective:

- (1) Low soil-nutrient availability = low cane yields = high purity = low percentage of nutrient in juices;
- (2) High soil-nutrient availability = high cane yields = low purity = high percentage of nutrient in juices.

Thus a low percentage in the juice would be apt to indicate a low availability in the soil, and a high juice content, a high soil availability. A low availability in the soil should result in low cane yields, which in turn would tend to have a higher purity, and this higher juice purity would most likely be associated with a low mineral con-

tent in such juice. Similarly a high availability in the soil should give high cane yields which would tend to have a low juice purity, and this low purity could be due to its high mineral content. Data showing major deviations from this relationship might be open to question and need further verification.

However, if it should be found desirable to adjust these percentages for differences in purity, such adjustment should be made from a basic purity of 87, which was the average juice purity of the data used in compiling these group classifications. Thus for purities below 87, the percentage standards used above would need an upward revision, while for purities above 87, these percentages would be slightly less than we have suggested.

#### STUDY No. 4

##### Juice Purity Relationships.

##### A. Relation of crusher juice purity to the per cent phosphate in the juice.

No. of Samples Averaged	Purity of Juice	Per Cent $P_2O_5$ in Juice
13	77.1 - 78.9	.048
17	79.0 - 80.9	.044
42	81.0 - 82.9	.046
113	83.0 - 84.9	.042
203	85.0 - 86.9	.036
260	87.0 - 88.9	.029
191	89.0 - 90.9	.027
28	Above 91	.028
<hr/>		
Total	867	

Comment: The relationship of phosphate in the crusher juice of cane to its purity is quite clear, i.e., with increased purity there was less phosphate in the juice.

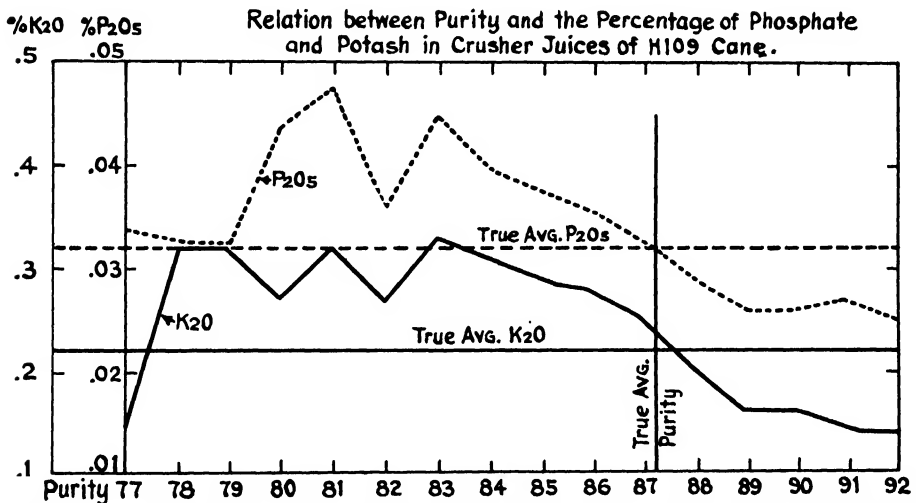
##### B. Relation of juice purity to the per cent potash of the juice.

No. of Samples Averaged	Purity of Juice	Per Cent $K_2O$ in Juice
13	77.1 - 79	.356
12	79.1 - 81	.331
48	81.1 - 83	.289
108	83.1 - 85	.294
211	85.1 - 87	.266
259	87.1 - 89	.199
178	89.1 - 91	.157
27	Above 91	.132
<hr/>		
Total	856	

Comment: As has been found in previous studies of this nature, we here note the association of a high potash content of crusher juice with the lower purities, and a low potash content with the higher purities.

C. Average phosphate and potash content of H 109 juices only, at different purities.

No. of Samples Averaged		Purity	Per Cent K <sub>2</sub> O	Per Cent P <sub>2</sub> O <sub>5</sub>
K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>		in Juice	in Juice
1	1	77	.150	.034
2	2	78	.317	.033
2	2	79	.318	.033
3	3	80	.265	.044
16	15	81	.321	.048
15	15	82	.274	.036
25	25	83	.330	.045
34	34	84	.312	.040
76	76	85	.287	.038
101	101	86	.278	.036
133	133	87	.248	.033
117	117	88	.203	.029
133	133	89	.164	.026
88	89	90	.156	.026
52	53	91	.138	.027
11	11	92 and above	.143	.025
Totals 809				
True Avg.		87.2	0.224	0.032



Comment: This study of the H 109 cane juices only, shows a direct relationship which has not always been generally recognized, i.e., the similar negative correlation between phosphate and potash with purity. Thus *both* phosphate and potash in the crusher juice show decreased amounts with the increasing purities, and vice versa.

D. Relation of phosphate in soil to quality ratio.

(Samples were included only if the potash in the same area was high.)

No. of Samples Averaged	P <sub>2</sub> O <sub>5</sub> in Soil	Q.R.
16	Low	7.8
13	Doubtful	7.0
2	Medium	8.0
114	High	8.7
Total 145		

*E. Relation of potash in soil to quality ratio.*

(Samples were included only if the phosphate in the same area was high.)

No. of Samples		
Averaged	K <sub>2</sub> O in Soil	Q.R.
40	Low	7.9
41	Doubtful	8.1
139	Medium	8.8
114	High	8.7
<hr/>		
Total	334	

Comment: There appears to be a direct relationship between the quality ratio of the cane juice and both the available phosphate and potash in the soil upon which the cane was grown.

## STUDY No. 5

## Available phosphate—cane tonnage relationships.

*A. Relation of phosphate in soil to cane yields.*(Only those samples which were *high in potash* are included in this table.)

No. of Samples		
Averaged	P <sub>2</sub> O <sub>5</sub> in Soil	Tons Cane per Acre
25	Low	68.2
9	Doubtful	73.6
2	Medium	72.6
134	High	85.4
<hr/>		
Total	170	

Comment: In those soils which were well supplied with potash, we note that (a) the heavier tonnages of cane are affiliated with the soils that show "high" P<sub>2</sub>O<sub>5</sub>, and (b) the poorer cane tonnages come from the soils that show a "low" phosphate supply.

*B. Relation of phosphate in soil to cane yields, in fields irrigated by pump water.*(Only those samples which were *high in potash* are included in this table.)

No. of Samples		
Averaged	P <sub>2</sub> O <sub>5</sub> in Soil	Tons Cane per Acre
16	Low	64.8
4	Doubtful	66.1
129	High	85.2
<hr/>		
Total	149	

Comment: In this group of fields which were irrigated with pump water and in which the supply of potash in the soil was high, we find that the larger cane yields are also associated with "high" phosphate.

*C. Relation between soil phosphate and cane yields, when the supply of soil potash is low.*

No. of Samples		
Averaged	Soil P <sub>2</sub> O <sub>5</sub>	Tons Cane per Acre
96	Low	71.7
51	Doubtful	78.5
11	Medium	68.1
39	High	72.3
<hr/>		
Total	197	

Comment: The effect of the availability of soil phosphate upon cane yields is not definite when the supply of soil potash is low. Potash is evidently a limiting growth factor.

## D. Relation of phosphate in the juice to cane yields.

(Samples were included only if the soil sample from the same area was *high in potash*.)

No. of Samples Averaged	Per Cent P <sub>2</sub> O <sub>5</sub> in the Juice	Tons Cane per Acre
8	Below .020	76.7
24	.020 - .029	80.7
36	.030 - .039	79.6
43	.040 - .049	88.3
25	.050 - .059	87.6
20	Above .060	78.6
<hr/>		
Total 156		

Comment: Generally greater cane yields are found here associated with crusher juices containing more than .04 per cent phosphate than where less than this amount is present.

## STUDY No. 6

## Available potash — cane tonnage relationships.

## A. Relation of cane yields to potash in soil.

(Only those samples which were *high in phosphate* are included in this table.)

No. of Samples Averaged	Tonnage Class	Tonnage Group	K <sub>2</sub> O in Soil Lbs. per Acre-Foot
7	35 - 40.4		286
8	40.5 - 45.4		394
9	45.5 - 50.4		297
12	50.5 - 55.4		223
Total 18 (54)	55.5 - 60.4	(Under 60 TCA)	463 (Avg. 333)
26	60.5 - 65.4		411
32	65.5 - 70.4		520
42	70.5 - 75.4		539
Total 29 (129)	75.5 - 80.4	(60 to 80 TCA)	510 (Avg. 495)
42	80.5 - 85.4		530
15	85.5 - 90.4		460
17	90.5 - 95.4		565
Total 14 (88)	95.5 - 100.4	(80 to 100 TCA)	602 (Avg. 539)
13	100.5 - 105.4		556
14	105.5 - 110.4		382
16	110.5 - 115.4		658
8	115.5 - 120.4		647
Total 4 (55)	120.4	(Over 100 TCA)	638 (Avg. 576)
<hr/>			
Total 326			

Comment: Where available soil phosphate was not a limiting growth factor, the larger cane tonnages were associated with the larger amounts of potash in the soil.

**B. Relation of cane yields to potash in the soil of fields irrigated by pump water.**  
(Only those samples which were *high in phosphate* are included in this table.)

No. of Samples Averaged		Tons Cane per Acre		K <sub>2</sub> O in Pounds per Acre-Foot	
	6	40 - 45.4		338	
	7	45.5 - 50.4		318	
	9	50.5 - 55.4		392	
Total	11 (33)	55.5 - 60.4	(Under 60 TCA)	360	(Avg. 352)
	20	60.5 - 65.4		389	
	20	65.5 - 70.4		455	
	34	70.5 - 75.4		549	
Total	23 (97)	75.5 - 80.4	(60 to 80 TCA)	578	(Avg. 493)
	32	80.5 - 85.4		568	
	12	85.5 - 90.4		498	
	13	90.5 - 95.4		613	
Total	11 (68)	95.5 - 100.4	(80 to 100 TCA)	530	(Avg. 552)
	47 (47)	Above 100.5	(Over 100 TCA)	645	(645)
<hr/>					
Total	245				

Comment: The larger cane yields were associated with the larger amounts of potash in the soil of this group of fields in which the supply of phosphate was high.

**C. Relation of soil potash to cane yields.**  
(Only those samples which were *high in phosphate* are included in this table.)

No. of Samples Averaged		K <sub>2</sub> O in Soil	Tons Cane per Acre
	38	Low	73.0
	42	Doubtful	68.2 (Avg. 70.6)
	142	Medium	77.4
	114	High	86.3 (Avg. 81.9)
<hr/>			
Total	336		

Comment: The average cane tonnage from the "high-medium" potash group is considerably greater than from the "low-doubtful" potash group.

**D. Relation between soil potash and cane yields, when soil phosphate is low.**

No. of Samples Averaged		Soil K <sub>2</sub> O	Tons Cane per Acre
	96	Low	71.7
	59	Doubtful	74.3
	129	Medium	77.2
	17	High	69.7
<hr/>			
Total	301		

Comment: When available phosphate was low, the influence of the supply of available potash on cane yields is not as clearly shown as when the phosphate supply was high.

*E. Relation of potash in juice to cane yields.*(Samples were included only if the soil sample from the same area was *high in phosphate.*)

No. of Samples Averaged	Per Cent K <sub>2</sub> O in the Juice	Tons Cane per Acre
4	Below .100	68.3
52	.100 - .200	73.4
95	.201 - .300	78.6
158	.301 - .400	81.7
18	Above .400	69.5
<hr/>		
Total	327	

Comment: Excluding the two extreme groupings, it would appear that the "Per Cent K<sub>2</sub>O in the Juice" had a direct influence on the cane yields.





## Some Aspects of the Internal Water Economy of the Sugar Cane Plant

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By H. A. WADSWORTH

Recent irrigation studies with the sugar cane plant in Hawaii (10) (13) have demonstrated that rate of cane growth, as ordinarily determined, is independent of soil moisture until the soil moisture has fallen to the permanent wilting percentage after which growth, as measured by elongation, is severely handicapped and soon ceases. Without more evidence than this, one is liable to draw conclusions which are not only unjustified but may be entirely misleading.

For example, since the plant at adequate soil moisture contents shows no sign of moisture scarcity in its growth response, one might conclude that sugar formation is proceeding at its maximum rate, an assumption which may or may not be true, but is certainly not demonstrated by the evidence. Or one may assume that the cells of the cane plant are completely turgid whenever the soil moisture is above the permanent wilting percentage but quickly shows signs of saturation deficit when the soil moisture falls below that critical value. Recent studies seem to indicate that such a conclusion is decidedly unsound.

The purpose of the present paper is to present the evidence which indicates that the sugar cane plant, grown in the hot, dry environment common to the irrigated areas of Hawaii, suffers a diurnal change in moisture content and to emphasize the significance that this inadequately appreciated phenomenon may have upon sugar formation.

### BRIX AS A MEASURE OF THE MOISTURE CONTENT OF CANE

The hand refractometer has long been popular as a device for measuring variations in the concentration of soluble material in the cell sap of cane. It is to be noted, however, that an increase in the refractive index of the solutions, which is what the device actually measures, may be due to either an increase in the quantity of sugar per unit mass of water, or to a decrease of water per unit mass of sugar. If it may be assumed that the actual amount of sugar in a specific joint is not subject to large and diurnal variations but remains practically constant over limited periods of time, the refractometer becomes a promising tool in measuring variations in the moisture content in the stick.

### BRIX OBSERVATIONS AT PIONEER MILL COMPANY, LTD.

Opportunities for testing the refractometer in this field of usefulness were offered by Pioneer Mill Company, Ltd., during the summer of 1935. Ten sticks in the upper ten lines of a watercourse plot were selected at random in the early morning. No stick so selected was subsequently discarded unless severe damage, from one cause or another, was noted during the sampling operation. Each stick

so selected was sampled for refractometer Brix in three places. The first of these was in the bottom third of the stick, the second in the middle third and the last in the top third and just below the lowest firmly attached leaf sheath.

The same procedure was followed late in the afternoon, ten sticks again being chosen at random. Sticks previously punctured were avoided.

The sequence outlined above was followed daily for the twelve days between July 15 and July 26 inclusive. The cane was variety H 109, about thirteen months old. The area sampled was in field B-4.

The results secured are tabulated in Table I.

TABLE I

Hand Refractometer Brix Readings, Morning and Afternoon Observations  
Variety: H 109  
Pioneer Mill Company, Ltd.  
Field B-4

(Reported Brix is the numerical average of 10 sticks  
selected at random in a small area)

Date 1935	—Bottoms—		—Middles—		—Tops—	
	AM	PM	AM	PM	AM	PM
July 15.....		19.7		18.1		15.6
16.....	18.4	18.4	17.3	17.7	14.7	14.8
17.....	19.7	20.0	18.9	17.3	14.8	15.5
18.....	17.5	19.2	16.4	18.3	14.1	14.9
19.....	18.7	20.3	18.1	19.7	15.4	17.1
20.....	19.2	19.4	18.9	19.3	17.2	15.9
21.....	17.8	18.3	17.2	18.2	15.0	14.3
22.....	17.7	20.3	17.1	19.8	14.7	17.0
23.....	19.4	19.6	18.1	19.5	15.5	15.8
24.....	19.1	18.9	18.7	18.5	16.8	15.2
25.....	17.7	18.5	16.9	18.2	15.2	15.8
26.....	19.3	19.6	18.7	18.8	15.9	17.7
Avg.....	18.6	19.4	17.8	18.6	15.4	15.8

If it be assumed, as suggested above, that the actual quantity of sugar involved remains constant, the averages in Table I indicate that the moisture content in the cane under observation suffers a diurnal fluctuation, being highest (Brix lowest) in the morning and lowest (Brix highest) in the late afternoon. Moreover, the difference seems greater in the bottoms and middles than in the tops.

A closer inspection of the data indicates that on only one day, July 24, was the Brix, in the bottom section, lower in the afternoon than in the morning. The same situation is to be noted in both middles and tops on the same day. It is interesting to note that a light shower was reported for that day. Attention is once more directed

to the physiological significance of light showers and dews upon the sugar cane plant (14).

As has been noted, only one day is reported during which the Brix in the bottoms declined during the day. But two such cases are noted for the middles and three for the tops. Apparently the cyclic relation suggested above becomes less positive as the point of sampling moves up the stick. The point is illustrated in Fig. 1, which is simply a graphical presentation of the data in Table I.

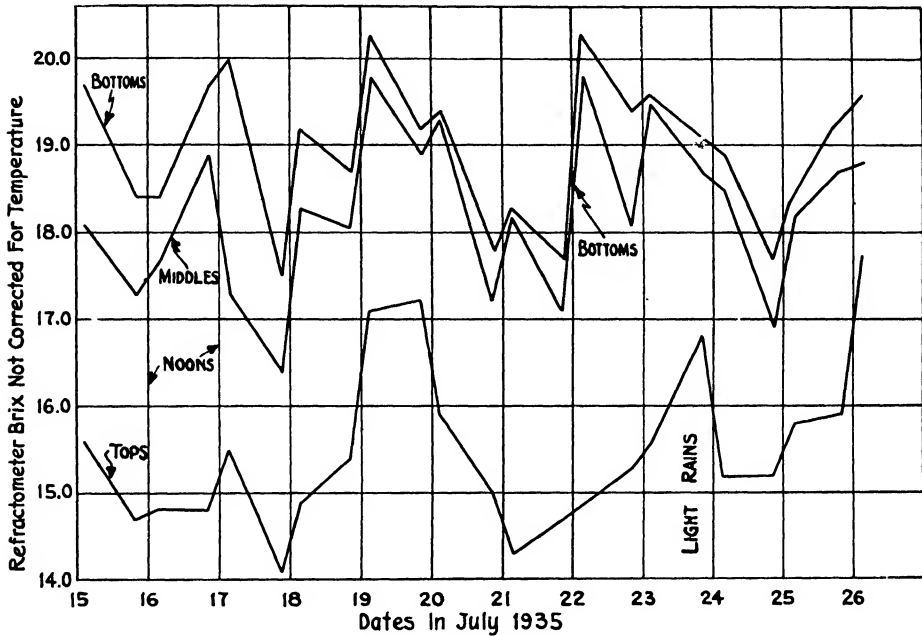


Fig. 1. Diurnal variations of Brix as measured with the hand refractometer. Variety H 109, Pioneer Mill Company, Ltd. Field B-4.

As has been said, each of the Brix figures given in the body of Table I is the average of ten individual random samples. Although this number is too small for convincing statistical attack, the 110 morning observations and 120 afternoon observations permit statistical scrutiny.

If again we assume that there is little or no increase in the soluble sugars present at the three points of observation, during the twelve days in question, we may average the entire number and equip each with some function of its variability. In this case, the old "probable error" is used.

When this is done, we can, within the limits of the correctness of the assumption given above, arrive at some measure of the significance of the differences reported. The results of these computations and the odds by which the significance of the differences may be judged are given in Table II.

TABLE II

Averages, Differences and Odds that the Differences are Significant from  
Summarized Brix Measurements in Table I

Part Sampled	Brix AM	Brix PM	Difference	Odds that Difference is Significant
Bottoms .....	18.6±0.11	19.4±0.09	0.8±0.14	7000:1
Middles .....	17.8±0.15	18.6±0.14	0.8±0.20	142:1
Tops .....	15.4±0.16	15.8±0.15	0.4±0.22	3.4:1

The odds given in the last column of Table II except in the case of "Tops" may be considered as highly significant. It is again to be noted that the moisture content cycle is most positive in the bottoms, less positive in the middles and least and possibly not significant in the tops of the canes.

It should be noted that the Brix figures purport to give the percentage of total solids in the solution on the refractometer plate. In order to convert the refractive index of the material into terms of Brix, a specific temperature must be selected as a standard and apparent readings must be reduced to this standard if reliable comparisons are desired. Browne's (3) refractometer table is based upon a temperature of 28 degree C.

Since the refractive index of sugar solutions decreases with increasing temperatures, it is apparent that uncorrected observations upon materials at a higher temperature than the standard will result in an apparent sugar percentage which is less than the real value, while observation upon materials at a lower temperature than the standard will give results that are greater than the true value.

Consequently the average Brix values secured in the afternoon must be increased, if we desire to form some basis of comparison since the temperatures in the cane were patently higher than 28 degree C. For similar reasons the morning averages would be reduced. It is evident that the differences reported in Table II are considerably less than the real differences. For obvious reasons, the probable errors for each of the averages would be practically the same after adjustment for temperature and the odds greatly increased.

#### OBSERVATIONS AT THE HILO VARIETY STATION

Similar observations made at the Hilo Variety Station failed to give convincing verification of the findings at Pioneer Mill Company, Ltd. Here both POJ 2878 and Yellow Caledonia were studied in accordance with the procedure already outlined.

The observations covered the period from August 2 to August 10, except for August 4. Both varieties were close to the Variety Station. The Yellow Caledonia was 16½ months old at the time of sampling; the crop was the first ratoon.

As in the case of the studies on Maui, each of the Brix figures reported is the average of ten individual samples. The details of the observations, except for the individual determinations contributing to the daily averages, are given in Tables III and IV.

TABLE III

Hand Refractometer Brix Readings, Morning and Afternoon Observations  
 Variety: Yellow Caledonia  
 Hilo Variety Station

Date	Bottoms		Middles		Tops	
	AM	PM	AM	PM	AM	PM
1935						
Aug. 2.....	18.9	19.3	18.7	19.2	19.7	18.3
3.....	18.6	18.6	19.0	19.9	18.7	17.6
5.....	18.8	20.1	19.4	18.5	19.3	18.0
6.....	20.2	19.7	19.9	19.8	19.5	18.9
7.....	19.5	20.0	19.5	19.3	19.6	19.6
8.....	19.0	19.4	19.1	19.2	18.5	18.1
9.....	19.5	18.8	19.5	19.6	17.7	18.4
10.....	19.8	19.8	19.5	18.9	19.0	17.7
Avg.....	19.3	19.5	19.3	19.3	19.0	18.3

TABLE IV

Hand Refractometer Brix Readings, Morning and Afternoon Observations  
 Variety: POJ 2878  
 Hilo Variety Station

Date	Bottoms		Middles		Tops	
	AM	PM	AM	PM	AM	PM
1935						
Aug. 2.....	20.8	20.3	21.6	20.9	20.0	20.1
3.....	19.3	19.1	19.7	20.1	18.1	17.1
5.....	19.3	20.1	20.7	18.5	16.5	18.0
6.....	19.5	19.9	20.3	20.1	15.8	20.6
7.....	19.7	20.3	20.6	20.6	19.9	19.5
8.....	18.8	19.6	20.0	20.1	16.3	18.8
9.....	20.1	20.0	20.6	21.2	19.2	20.8
10.....	20.5	19.7	21.4	20.7	20.8	19.6
Avg.....	19.8	19.9	20.6	20.3	18.3	19.3

With neither Yellow Caledonia nor POJ 2878, under conditions as they existed, was there any significant difference between the uncorrected morning and afternoon Brix readings. No observations were made upon temperature at the time of observation. Brown (3) states that in this bracket of concentrations a change of temperature of one degree Centigrade results in a change of Brix of 0.07 per cent. Hence a total difference in temperature between the morning and afternoon reading of 5 degree C. would increase the difference of Brix by 0.35 per cent. As has been indicated, this correction increases the difference between the morning and afternoon samples without modifying the probable error of the difference. Whether this correction would show significant differences in the Brix with these two varieties at Hilo cannot be determined without definite data as to the temperatures involved.

It should be noted that frequent rains may have added their effect to the sequence which might have been expected from the Maui results.

## OBSERVATIONS AT WAIPIO, OAHU

More detailed observations at the Waipio Substation tended to strengthen the impression that H 109 at least, when grown under irrigated conditions, seemed to enjoy a diurnal fluctuation of cell sap concentration.

In these series of tests\* fifteen individual joints were sampled with the hand punch and flagged in such a way that the same joints might again be sampled as a measure of modifications in the concentration of materials in solution.

This first series consisted of observations on the evening of October 3, 1935, and the morning of October 4. Care was taken to time the periods of observation so that the morning temperatures and afternoon temperatures might be as similar as possible. In addition to the apparent Brix readings for each of the observations, Table V gives the temperature at the time of making each of the Brix determinations.

TABLE V

Hand Refractometer Brix Measurements on Fourteen Selected Joints of  
H 109 Cane, Age 12 Months, Waipio, Oahu

Joint	Morning October 3		Afternoon October 4	
	Brix	Temperature	Brix	Temperature
1.....	14.8	23.4°C	15.0	26.6°C
2.....	17.6	23.6	17.3	26.2
3.....	14.5	23.8	14.4	25.9
4.....	16.0	23.6	16.7	25.7
5.....	13.2	23.6	14.0	25.8
6.....	17.1	24.6	17.6	25.6
7.....	15.8	24.6	15.3	25.4
8.....	16.8	24.7	17.1	24.8
9.....	16.6	25.0	17.6	25.2
10.....	15.6	25.0	16.3	25.7
11.....	13.8	25.0	15.4	25.6
12.....	12.8	25.0	13.8	25.8
13.....	14.0	25.0	14.0	25.5
14.....	14.1	25.0	14.2	25.5
Avg.....	15.2	24.4	15.6	25.7

Since the same joints sampled in the morning are sampled again in the afternoon, we have 14 pair of observations which may be compared by Student's method. When this procedure is used, the odds are more than 50:1 that the difference is significant.

In order to guard against the possibility of having the observations of the second series influenced by the punctures in the joints made for the previous series, a second set of observations was planned.

In this case the observations of the first series on the selected joints were made late in the afternoon of October 12, 1935. The second series was run on the following morning. Here the differences were much more marked; again the afternoon readings exhibited a higher Brix than the morning.

Details of observations are given in Table VI.

\* The writer is indebted to Ernest Johnston and Caleb Burns, Jr., students at the University of Hawaii, for assistance in making these observations.

TABLE VI

Hand Refractometer Brix Measurements on Fifteen Selected Joints of  
H 109 Cane, Age 12 Months, Waipio, Oahu

Joint	Afternoon October 12		Morning October 13	
	Brix	Temperature	Brix	Temperature
1.....	20.1	24.1°C	15.9	21.8°C
2.....	14.5	24.2	13.2	21.7
3.....	15.2	24.2	13.5	21.6
4.....	13.6	24.1	12.3	21.6
5.....	13.0	24.1	12.0	21.6
6.....	15.8	24.2	13.8	21.7
7.....	16.0	24.3	15.0	21.7
8.....	15.3	24.2	12.6	22.0
9.....	9.5	24.1	8.4	22.0
10.....	18.8	24.1	18.1	21.8
11.....	17.5	24.0	16.8	* 21.8
12.....	11.8	24.5	9.7	22.4
13.....	15.0	24.4	14.1	23.1
14.....	16.9	24.3	15.8	23.0
15.....	15.4	24.3	12.9	22.8
Avg.....	15.2	24.2	13.6	22.0

Here the numerical difference between the average afternoon and morning Brix is greater than in the previous series. The odds, again by Student's method, are much more than 100:1 that the difference is significant.

Although some care was given to starting the two runs at such times that the temperatures would be equal during the two runs, this end was not gained. In both cases, the afternoon temperature was greater than in the morning. If this were allowed for and corrections applied as suggested by Browne (3), the differences and consequently the odds would be somewhat increased.

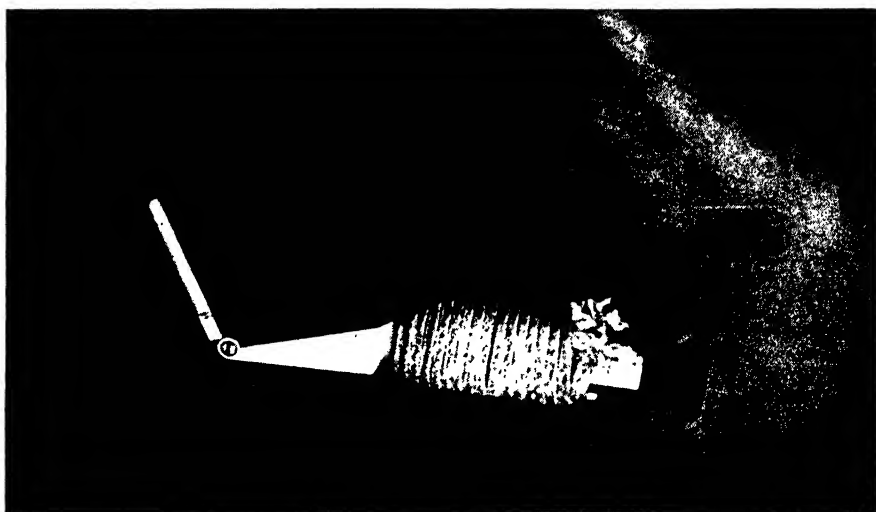


Fig. 2. The hand refractometer insulated with asbestos to protect it from the effect of body temperature.



Every care was used to get results that were accurate within the capacity of the instrument. The plate was flushed with distilled water after every observation and wiped dry with a clean cloth. An insulating sleeve of asbestos yarn was attached to the barrel of the refractometer to protect the device from the body heat of the operator. The device with its insulation is shown in Fig. 2.

#### DETAILED GROWTH MEASUREMENTS

Since such fluctuation of moisture content in the stick should logically find some expression in growth response, a series of growth measurements were made at Pioneer Mill Company, Ltd., concurrently with the Brix measurements already noted. The usual procedure was slightly modified.

In the usual procedure with growth studies (10) (11), twenty sticks are permanently selected and daily measurements made upon the distance between a permanent datum and the last visible ligule or "dewlap."

During the present measurements, this procedure was followed except that observations were made twice a day. One series of measurements was made during the early morning and another at about sundown. Moreover, observations on the growth history of the leaf spindle were made twice daily. This was accomplished by establishing a fixed point, by means of India ink on the innermost leaf in the leaf spindle and measuring from this point to the corresponding datum point. Consequently, we have two measures of the growth performance of the twenty sticks under consideration in considerable detail. It should be mentioned that the twenty sticks under measurement were in the middle of the area being sampled for Brix.

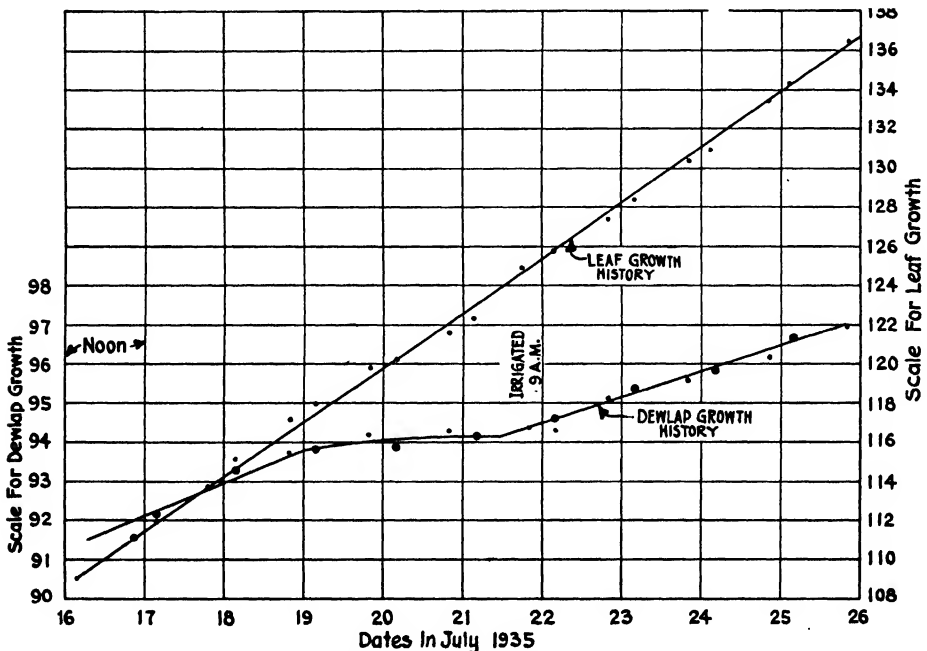


Fig. 3. Results of growth measurements at Pioneer Mill Company, Ltd. Note the typical growth history of the dewlap. Of particular interest is the uniform advance of the leaf bundle after elongation of the stem below the dewlap has ceased.

The details of these average measurements are shown in Fig. 3. The lower curve giving the growth history of the "dewlap" is typical of those reported from Waipio (10) and Waiialua (11). Here the growth is at approximately a uniform rate until soil moisture becomes a limiting factor. After a short transition curve, growth is resumed after the irrigation noted.

The addition of a second series of points during each twenty-four-hour period gives some evidence that growth is extremely slow during the daylight hours when soil moisture is low. In fact, on the afternoons of July 20 and 21, there seems to be an actual shrinkage of the tissue. How real this shrinkage is cannot be determined from the evidence at hand. But it seems apparent that the rate of growth is significantly different during the day and night at low moisture contents.

Quite a different picture is presented by the growth history of the leaf bundle: Here the datum point on the leaf seems to move uniformly and quite independently of soil moisture depletion within the limits covered by the Pioneer observations.

Similar observations on the plants growing in small tanks at the Experiment Station in Honolulu show similar results. Here the plants were younger and being grown in containers permitted approximations of the relative soil moisture content by weighing.

The growth and soil moisture histories for Tank No. 1 are shown in Fig. 4. Here the gross weight curve which is a measure of the rate of soil moisture depletion shows its customary form with a weight of 164 pounds being associated with a

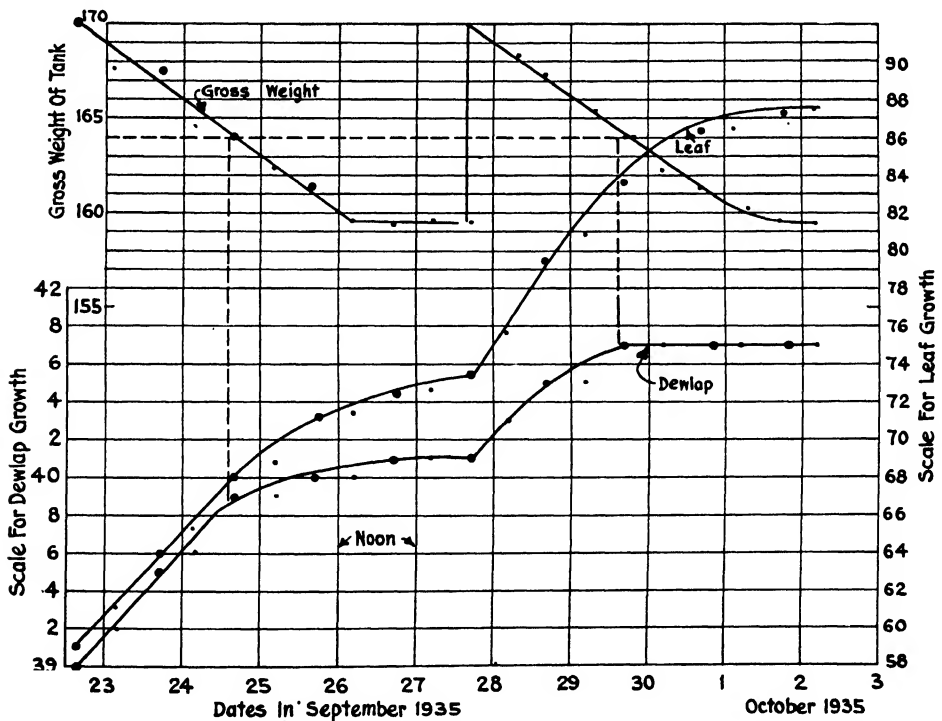


Fig. 4. The growth history of H 109 cane grown in small tanks at the Experiment Station H.S.P.A. Here the leaf bundle continues to elongate for some time after the permanent wilting percentage has been reached.

physiological disturbance which has resulted in a marked reduction of the rate of growth as measured by dewlap measurements. It is to be noted again that the spindle continues to grow for some time but at a reduced rate. Here, too, it is evident that during periods of low soil moisture contents growth as measured by both dewlap and leaf extension is greater by night than by day.

It should be noted that the plants reported in Fig. 4 were seven months old in September ; only a short section of millable cane was visible.

## DISCUSSION

The suggestion that the cane plant grown in the hot, dry environment of most of the irrigated plantations of Hawaii suffers diurnal variations in moisture content is not without support from workers with other plants. Batholomew (2) reports that the leaves of the lemon tree are often kept turgid at the expense of moisture stored in the fruit. After reporting a long series of observations in detail, Batholomew states, "The records show that during periods of excessive evaporation there may be not only a daily water deficit but one which may last during the night as well as during the day for at least three or four weeks at a time. That such a deficit must have a profound effect upon the fruit would appear to be evident." Although such conditions might adversely affect the quality of the lemon fruit, it is not clear how such deficits might affect the sucrose content of sugar cane.

Livingston and Brown (8), working with desert plants near Tucson, Arizona, report large variations in the moisture content of leaves during a twenty-four-hour period. In these studies a variety of pigweed (*Amaranthus palmeri*) showed a reduction of maximal leaf moisture of 39 per cent during the heat of the day while mesquite (*Prosopis velutina*), the most constant species studied, showed a reduction of 14.3 per cent.

Maximov (9) gives a comprehensive survey of work in this field and adds the following comment, " . . . the suction developed in wilting leaves—provided that the plant wilts sufficiently slowly and deeply—is transmitted to all parts of the plant organism. Under these conditions the transpiring leaves draw upon such reserves of water as the other organs may contain."

Krasnoselsky-Maximov, reported by Maximov (9), is more specific. Mme. Maximov noted diurnal variations in leaf moisture similar to those reported by Livingston and Brown. But she adds that, "These fluctuations naturally vary from day to day. On hot days with high moisture deficit in the air (that is low relative humidity) the fluctuation was more pronounced; while on cooler days they were less perceptible." Attention is again directed toward the relative rate of supply of moisture by the roots and the rate of demand by the atmosphere (15).

More recently new devices developed by Bachmann (1) have permitted the precise measurement of leaf thickness and leaf area during these diurnal variations in leaf moisture. Such measurements show a decided correlation between leaf thickness as well as area, and moisture content.

Recent work by Hartt (7) shows that the same processes are evident with sugar cane. Dr. Hartt determined the moisture contents of leaves, sheathes and green-leaf cane (H 109) after a long period of darkness during which full turgidity was

presumably restored and after five hours of bright sunlight and high temperature. Two series of such tests were run. In one of these, the plants enjoyed abundant soil moisture; in the other, the moisture content was unquestionably below the permanent wilting percentage.

Dr. Hartt's results in a modified form are given in Table VII.

TABLE VII

Moisture Contents of Varying Parts of the Cane Plant After Different Exposures

	Per Cent Moisture			Per Cent Moisture	
	Time of Sampling			Time of Sampling	
	8 AM	1 PM		8 AM	1 PM
High Soil Moisture:			Low Soil Moisture:		
Leaves .....	70.7	69.6	Leaves .....	67.9	66.6
Sheathes .....	85.2	84.2	Sheathes .....	79.6	76.3
Green-Leaf Cane ..	88.5	87.8	Green-Leaf Cane ..	84.4	84.1

The decreases in moisture contents, with exposure to the high evaporating capacity of the greenhouse, are not as great as those which have been noted in the literature. But the modifications of moisture contents are significant in all cases when considered in conjunction with their probable errors as given by Dr. Hartt. Moreover, it should be noted that only five hours elapsed between the two times of sampling. It is highly probable that the reduction of moisture in blades, sheathes and stems would continue in the afternoon, resulting in a diurnal variation much greater than that reported.

It should be noted that evidence of this variation in moisture content in the cane plant has been reported only from areas in which the evaporating powers of the atmosphere during the day are great. Whether or not the efforts to trace such diurnal variations at Hilo failed to give significant results because of an insufficient demand upon the aerial parts of the plant during the day or because of the different varieties in use there cannot be determined from the present data. If the first alternative is involved, the significance of a wide range of temperatures between day and night is apparent, as has been suggested by Das (4).

If it be assumed that, under the hot, dry environments of irrigated plantations in Hawaii, the sugar cane plant suffers a diurnal variation in moisture content, one is tempted to speculate upon the relation of this phenomenon upon sucrose formation.

Considerable basis for such speculation is to be found in the literature. For example, Spoehr (12) demonstrates that, with cactus, a lowering of the moisture content in the plant is associated with an increase in polysaccharides and a reduction in monosaccharides. Das (5) in a thesis presented to the University of Minnesota states that, "if the cells are high in water, the elaborated carbohydrates move toward the complex types, on the other hand when cells are low in hydration the carbohydrates move toward the soluble type and in cane, accumulate as sucrose." And again by correspondence Das (6) states that, "we have evidence that seasonal variations in the sucrose content of even the dry-leaf section are associated with changes in the water content." Hartt (7) has discussed the significance of moisture content

of cells of the sugar cane plant, upon the physiological processes underway on the basis of the conditions required for the chemical processes which might be expected.

It is recognized that other conditions than varying moisture contents are of great importance in the general problems of sucrose formation and storage. Of these the enzymatic equilibrium is perhaps the most important. A discussion of this phase of the problem is outside the scope of the present paper. It may be said, however, that Spoehr (12) discusses the relation between this equilibrium, temperature and moisture content in some detail.

Such experimental results, as those presented, are suggestive rather than conclusive. The need for more evidence is apparent not only toward the end of verifying the tentative results presented but also in the interpretation of such results in the laboratory.

If any credence can be given these results and the speculation based upon them, one is impressed with opinions and practices in the industry which have been based upon observations and field experiments. For example, it used to be said that it did the cane crop good to dry out. At Oahu Sugar Company, Ltd., and at Waianae Company, a practice of "tapering off" the irrigation interval prior to harvest has resulted in high sugar yields although such a practice should not, from our present conceptions, increase cane tonnage. Moreover, the old determinations of optimum irrigation intervals resulted in the use of intervals from three to eight days longer than those determined by cane growth studies.

The statement has frequently been made that the inner leaf bundle of sugar cane rarely shows signs of wilt early in the morning regardless of soil moisture. It seems possible, at least, that the moisture required for this restored turgor may be translocated from other parts of the plant as Maximov (9) has suggested.

#### SUMMARY

Some evidence is present that the moisture content of H 109 cane, grown under irrigated conditions, suffers a diurnal variation of considerable magnitude. Such moisture content is higher at daybreak and lower at nightfall.

Such determinations were made by use of the hand refractometer, it being assumed that variations in Brix might be more accurately charged to variations in moisture than in soluble material.

An attempt to duplicate the results with other varieties (POJ 2878 and Yellow Caledonia) at Hilo Variety Station failed to give significant results unless an unconvincing temperature correction is invoked.

There is some speculation as to the role of this daily moisture fluctuation and sugar formation.

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## The Effect of Nitrogen on Cane Yield and Juice Quality\*

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By U. K. DAS AND A. H. CORNELISON

In the course of the last fifteen or twenty years most of the sugar plantations of these Islands have substantially increased, and some have actually doubled, their per acre yields of sugar. Various factors have contributed; important among these is the increased use of nitrogen. During the same period, however, the quality of juice has steadily gone down—the decline amounting in some cases to nearly fifteen per cent. Some of us with a bias for figures have calculated that if we had the present tonnage of cane together with the old time quality, the sugar production per acre, and hence our margin of profit, would be considerably greater. An attractive prospect indeed, marred only by the solemn warning of the sceptic that we may not eat our cake and have it too, or something to that effect. However, the proposition seemed so attractive that we felt it would be worth while to inquire if quality of cane and quantity were by nature opposed to each other; that is, if it would not be possible to have the increased tonnage without losing so much of the quality.

The problem we set out to answer in this preliminary experiment was, "How does nitrogen affect yield and quality of cane?" Surely without such knowledge all our efforts to improve quality will be like shooting at a target in the dark. We may hit it but the chances are greater that we shall miss it.

We felt that one satisfactory way to approach the problem would be to study the effects on yields and quality of three different amounts of nitrogen applications—a low application such as was used a decade or so ago, a medium or standard application such as is used at present on the irrigated fields of Oahu, and a very high application such as our past experience indicates to be entirely too much. Actually the amounts we used for this study were 133, 266, and 645 pounds per acre respectively. The nitrogen was applied in seven doses, the last dose going in on the fourteenth month. In each series we applied the same amount of phosphate and potash fertilizers, the same amount of water and a sufficient quantity of it, so that the differences in yield or quality of cane would be due to amounts of nitrogen alone.

The cane was H 109 planted in three blocks—one each for the low, the medium, and the high series. Beginning at four months and every two months thereafter we harvested one line of cane from each block, took careful weights, crushed the juice and determined sucrose and glucose. We also made many other chemical determinations. There were two good reasons for harvesting the cane at different ages, one was to find out if the differences due to treatment lasted through the entire life of the crop, or disappeared as soon as we stopped fertilization, and the second was to see if the treatment differences were repeated from harvest to harvest. In the following pages, we are reporting the results of eleven such harvests—the last one being at the age of 24 months.

Only too often we find it difficult to compare one series of planting with another because the original stand was different. To eliminate this difficulty we planted a

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\* A more technical account is to appear in the April 1936 number of *Plant Physiology*.



very large number of seed pieces and after germination kept the same number of mother stalks (i.e., stalks that arose directly from the seed piece) in each line in the three treatments. This number was 65 for the 25-foot lines used or 2.6 stalks per running foot. Our past experience had indicated that probably that was all that H 109 cane could carry per foot. (Here we might mention that the results of this experiment show that as many as 3.2 stalks of H 109 can be grown on one foot of line.) Furthermore, we were interested in studying how many daughter stalks and grand-daughter stalks (suckers) would come up, so we tagged all these mother stalks in order that at no time we would have trouble in identifying these canes in the field.

#### COLOR, WIDTH, LENGTH AND LONGEVITY OF LEAVES (FIG. 1)

We found that as the nitrogen application was increased, the color of the leaves became darker green to almost blue-green. In the low treatment the leaves appeared pale and yellowish. The difference in color was more marked around ten to twelve months. After the nitrogen application stopped, the color differences slowly began to disappear.

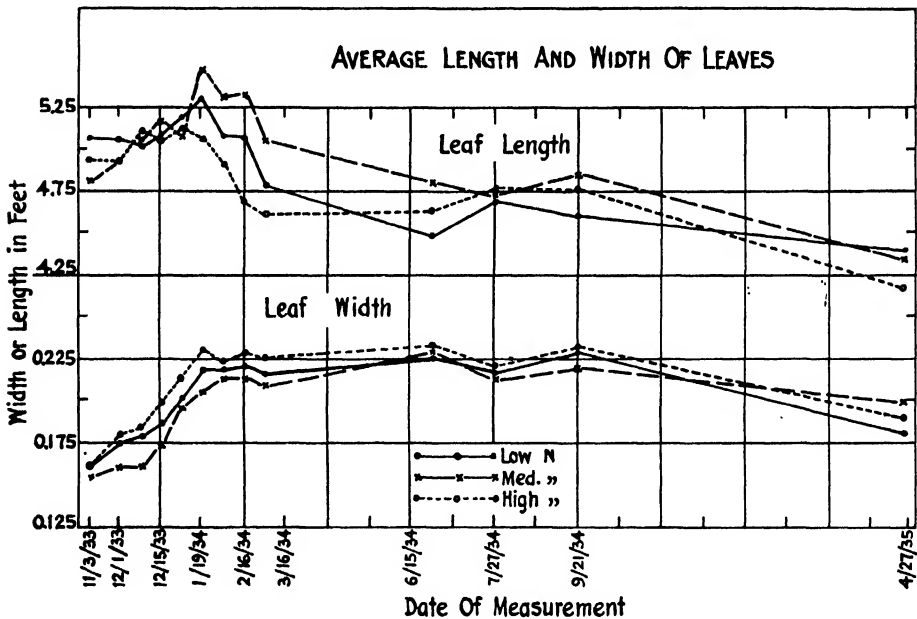


Fig. 1

In general, the higher the amount of nitrogen the wider are the leaves, although the same thing cannot be said with certainty of the length of leaves. Interestingly enough, the leaves formed at the "boom stage" were wider and larger than those formed at any other time. After the application of nitrogen stopped, the leaves began to be narrower and shorter, like those of cane growing on poor soil areas.

We found that generally a leaf remains green for about 2 to 2½ months from the time it opens out fully. In other words that which is young, hardly millable top today becomes a part of the dry-leaf cane in about two months.

The higher the amount of nitrogen applied, the greater the number of leaves formed in an interval of time. In our high series we had 41 new leaves per stalk between November 1934, and October 1935, and 37 in the low series in the same period of time, giving us a difference of about 11 per cent in favor of the high series. We know that each leaf is carried on a separate joint, so we conclude that the more nitrogen applied the greater the number of joints of cane formed.

#### STAND OF CANE

Another striking difference was in the matter of stand. The canes in the high series started lodging as early as six months, those in the medium at about eight to nine months, while many of the stalks in the low series were still erect around fourteen to fifteen months. People working with cereal crops have found that early lodging is often due to poor development of roots and of the woody supporting tissue. We believe that such is very likely the case even with sugar cane.

#### LENGTH GROWTH (FIG. 2)

To determine if the differences in treatment showed in the rate of length increase, we selected twenty mother stalks from an inside line and measured their length at two-week intervals. From these measurements we calculated the rate of increase in length. For the first few months there was not much difference in this respect between the cane in the three series. However, from about the seventh month on, the differences began to be quite marked—the more nitrogen the greater the elongation. Rather significantly, there was a great difference between the low

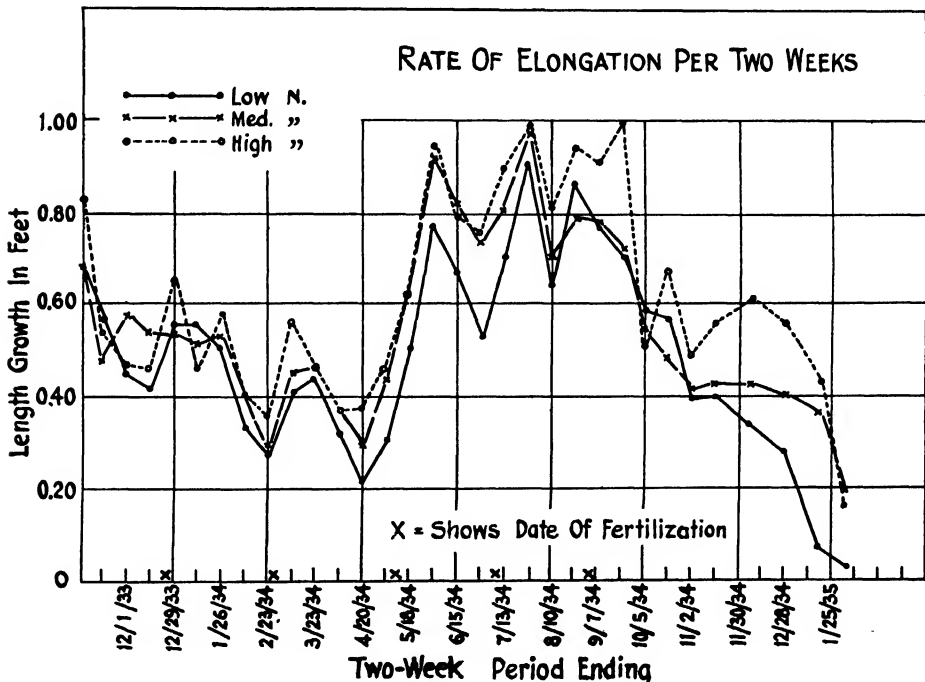


Fig. 2

and the medium series, but only a very small difference between the medium and the high although the amount of nitrogen applied was nearly twice as much in the latter as in the former series. This would show that by going very much beyond our standard practice we may use a lot of fertilizer, but we are likely to get very little extra growth (and tonnage, as we shall see presently).

From time to time we have stated that cane growth and temperature show a close correlation. This study further proves that point. We find that as long as cane is receiving plenty of plant food and water, rate of elongation is directly proportional to our day-degree (which we have defined in the past as degrees Fahrenheit of mean maximum temperature above 70 degrees F.) In other words, we find that if 75 degrees F. of mean maximum temperature for two weeks gives an increase in length of say one foot, then 80 degrees F. for the same length of time increases the cane length by two feet, because our total day-degrees are as 5 is to 10, i.e., as 1 is to 2.

#### SUCKERS—DAUGHTER STALKS AND OTHERS (FIG. 3)

Not only did we tag the mother canes but we also tagged all the suckers that came up within the first three months. We shall call these suckers the daughter stalks. At harvest the mother, the daughter, and all the other stalks were separately weighed. If we now take the weight of all the cane in the line as 100, we find in our experiment that the mother stalks weighed about 80, the daughter stalks 15 or more. This goes to show that up to the time of our last harvest at 24 months, the late suckers were not of much account. Probably such would not be the case under a different set of conditions or with another variety.

We further found that the more nitrogen applied the more numerous were the daughter stalks and other suckers.

#### MORTALITY OF CANE (FIG. 3)

From about the sixteenth month on, we found at each harvest that a certain number of the mother stalks had dead or dying tops. This was more noticeable in the high-N series than in the medium or the low. This greater top mortality may be due to the greater crowding, and greater plant competition in the high-N plot than in the others. In the high series we found heavy borer damage; this also might have had something to do with greater mortality. (Our entomologists tell us that the extremely heavy trash blanket in the high series made it difficult for the borer parasites to get to the borer larvae to parasitize them.) At the rate the mortality was mounting, it looked as if we would have no live mother stalks at the end of thirty months or more.

#### YIELD OF CANE (FIG. 4)

We have already mentioned that we had one harvest every two months from the fourth month on. Each time we separated the non-millable top from the millable cane. We then cut the millable cane into two parts: (1) the dry-leaf cane, i.e., the part of the cane where the joints are fully exposed and the leaves are either dried up or have fallen off, (2) the millable green-leaf cane where a few wholly or partly green leaves are still attached. The dry-leaf cane is what we usually term the ripe

# WEIGHT OF FIRST-AND SECOND-ORDER STALKS AS PER CENT OF TOTAL WEIGHT

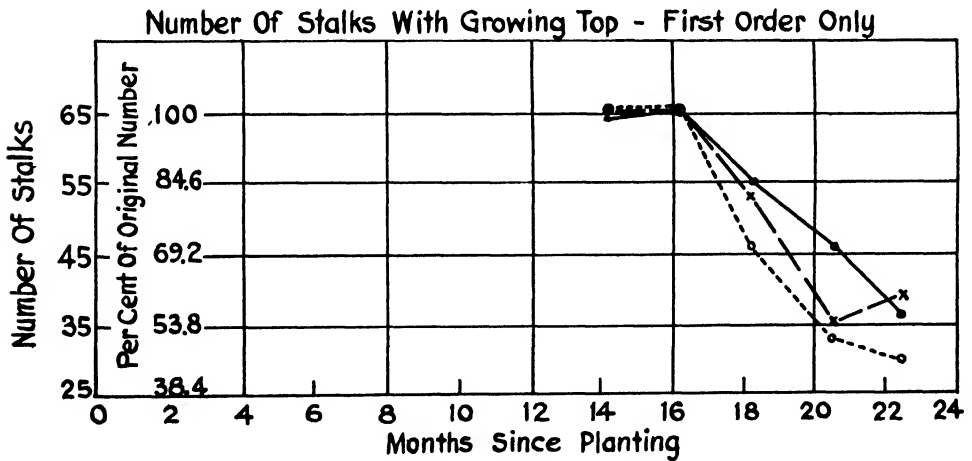
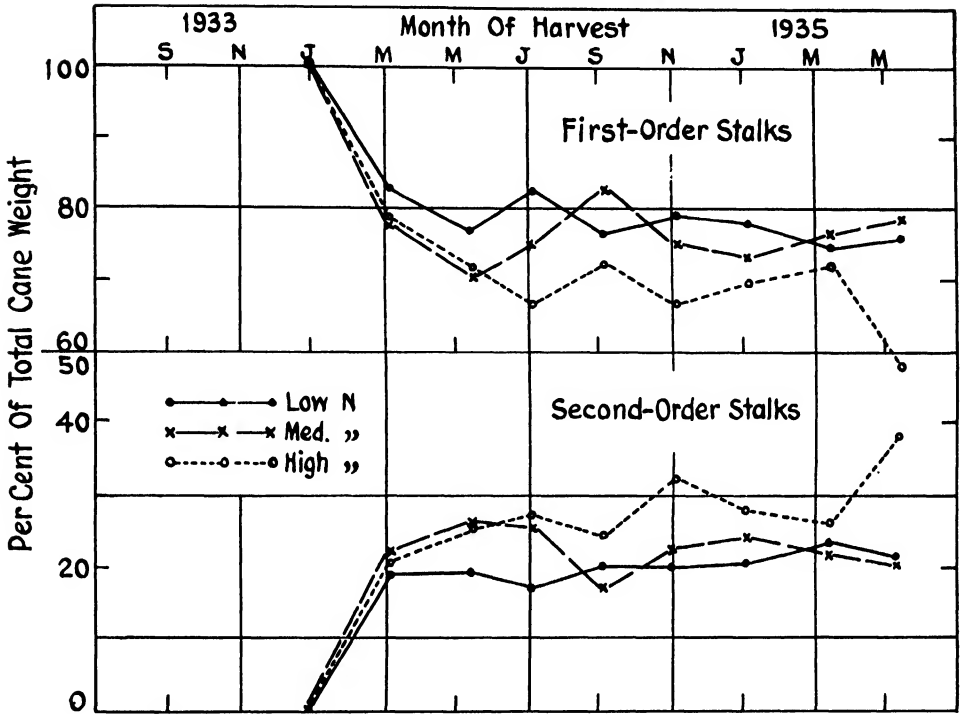


Fig. 3

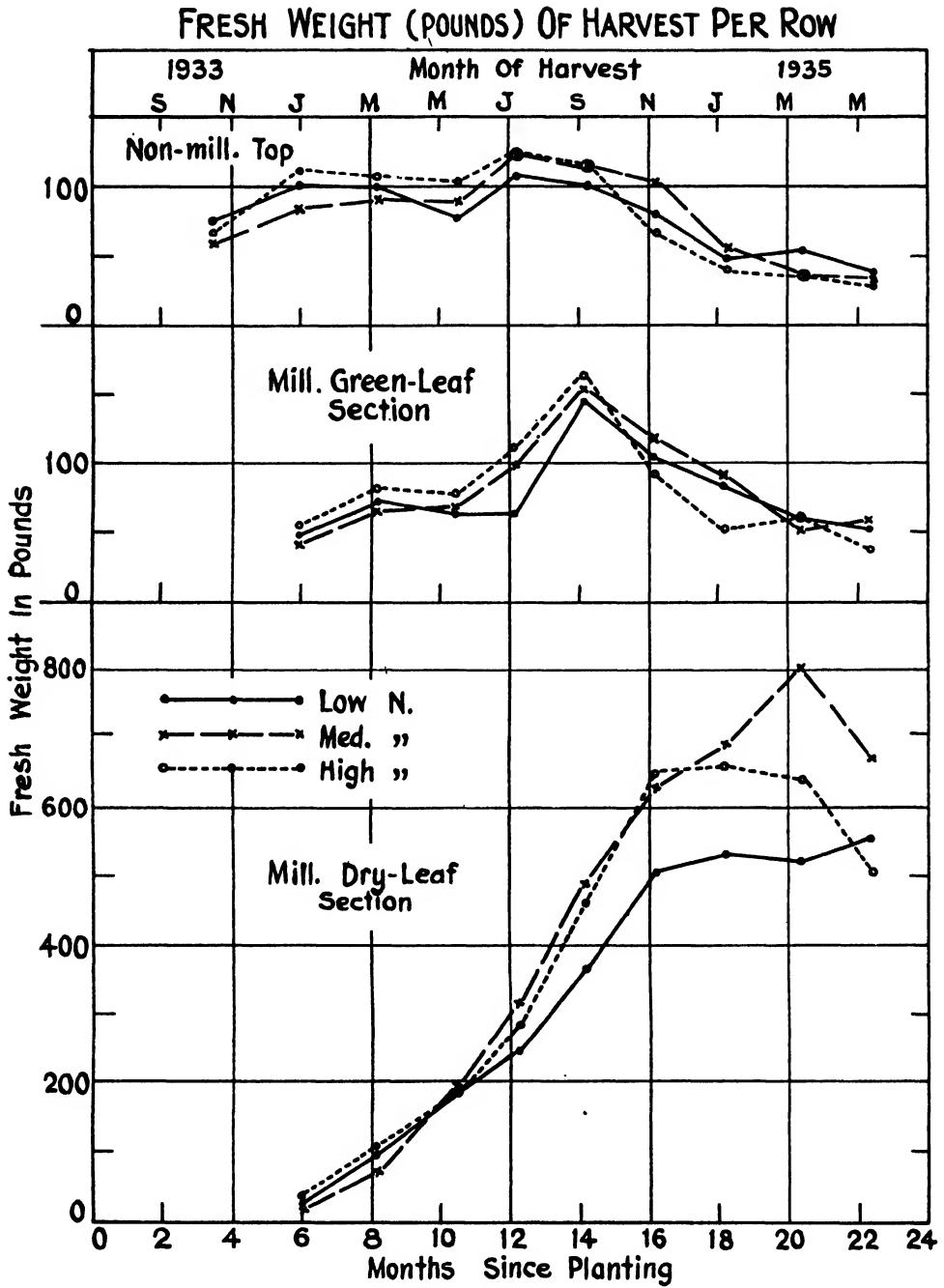


Fig. 4

part of the cane while the green-leaf part is where the juice is not quite as good as it might be.

We found that the more nitrogen applied the more was the weights of the tops, the millable green-leaf and the dry-leaf sections. However, the picture changed a little beginning at the sixteenth month because of great mortality in the high series. It might appear as if in this series we had really given the cane more than was good for it. As a result, in the latter months the medium series showed the highest yield, indicating that our standard practice was probably about right—certainly better than the two extremes of fertilization here given.

That which is the leafy top today becomes a part of the millable stalk in two months and that which is the green-leaf section becomes a part of the dry-leaf section. Therefore the top and the green-leaf sections in the succeeding harvests represent new material. The dry-leaf section on the other hand shows an accumulative effect, for it contains old growth as well as new additions.

We found that for a while the weight of the dry-leaf cane was about the same in all the three series. Then, the low series began to fall behind and stopped growing entirely almost immediately after the nitrogen application was stopped, indicating that the cane in this series was really living, one might say, a hand-to-mouth existence. Surprisingly enough the medium series proved to be decidedly better than the high, especially so in the latter months after mortality started in the high series.

However, when we added the weight of all kinds of stalks including the daughters and the later suckers, we found (Fig. 5) that the high series was slightly better than the medium in cane tonnage up to about the sixteenth month, although this difference was not at all in proportion to the difference in the amount of fertilizer applied. We note with interest that we could very well have predicted this result from our growth measurements. There too, it will be recalled, we found little difference between the medium and the high series.

Summarizing the yield data we may then say that increasing the nitrogen application beyond a certain optimum point will not increase the yield to any extent; in fact, it might even lower the yield by injuring the cane.

### JUICE QUALITY

When we speak of juice quality becoming poorer as a result of heavy nitrogen fertilization, we usually have in mind the concentration of sucrose in juice. This concentration is actually the result of two things—the amount of sucrose present and the amount of water present. Thus we may have the same amount of sucrose in two containers—say, two plants—but if the amount of water is different in the two, the concentration of sucrose will be different. Again, we may have actually more sucrose in one plant than in the other but due to greater water content, the concentration might still be less in the first than in the second. We can thus easily see that it is not always safe to think in terms of concentration, if what we are after is the amount of sugar. (True, the greater the dilution of sucrose the greater the cost of manufacture—but we shall not consider the question of cost for the present.) The question then, we asked ourselves, is this, “Does the observed difference between the poorly and the highly fertilized plants merely indicate concentration dif-

# WEIGHT OF MILLABLE CANE AND\*ALL FRESH MATTER\* FROM ALL ORDERS OF STALKS

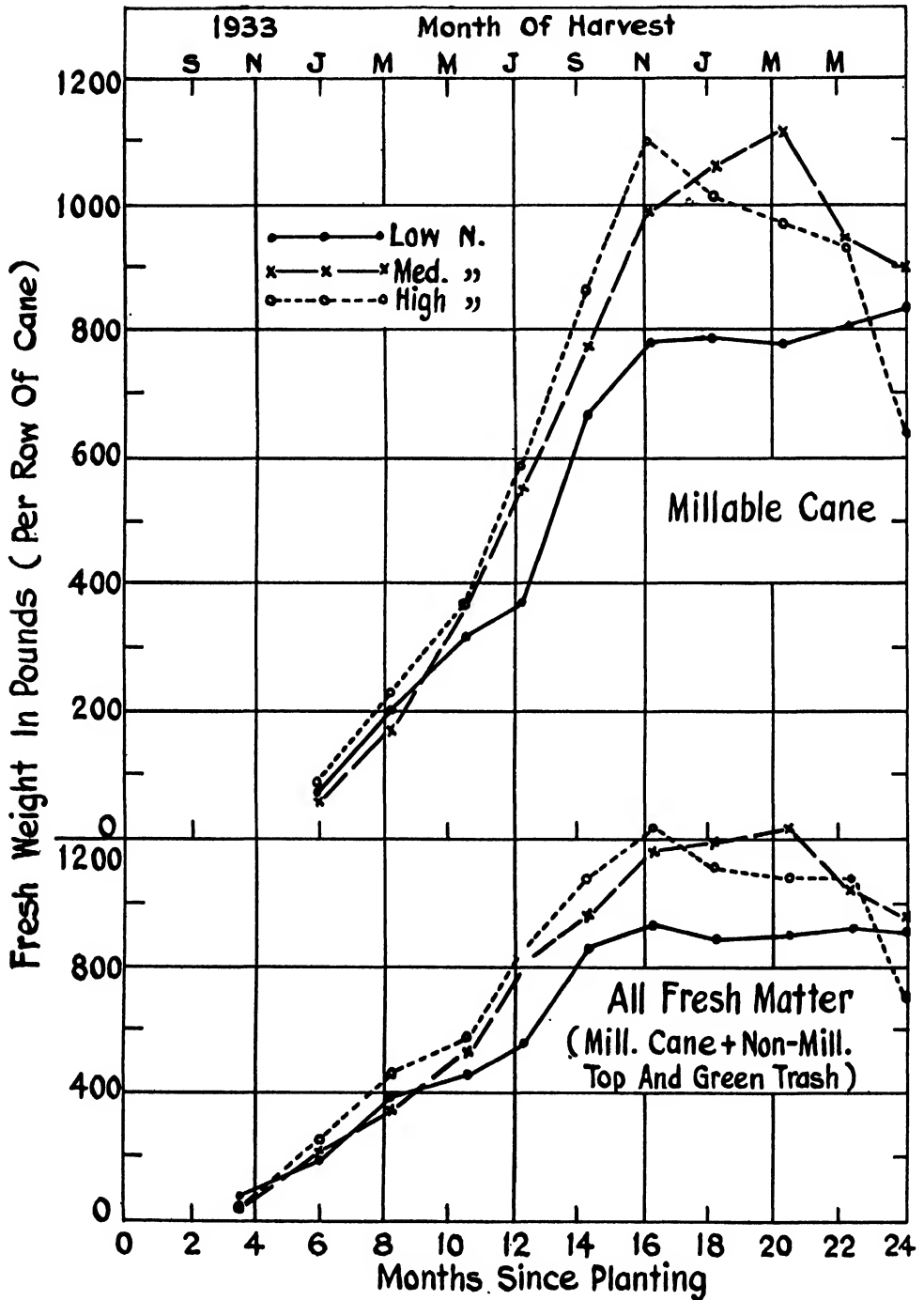


Fig. 5

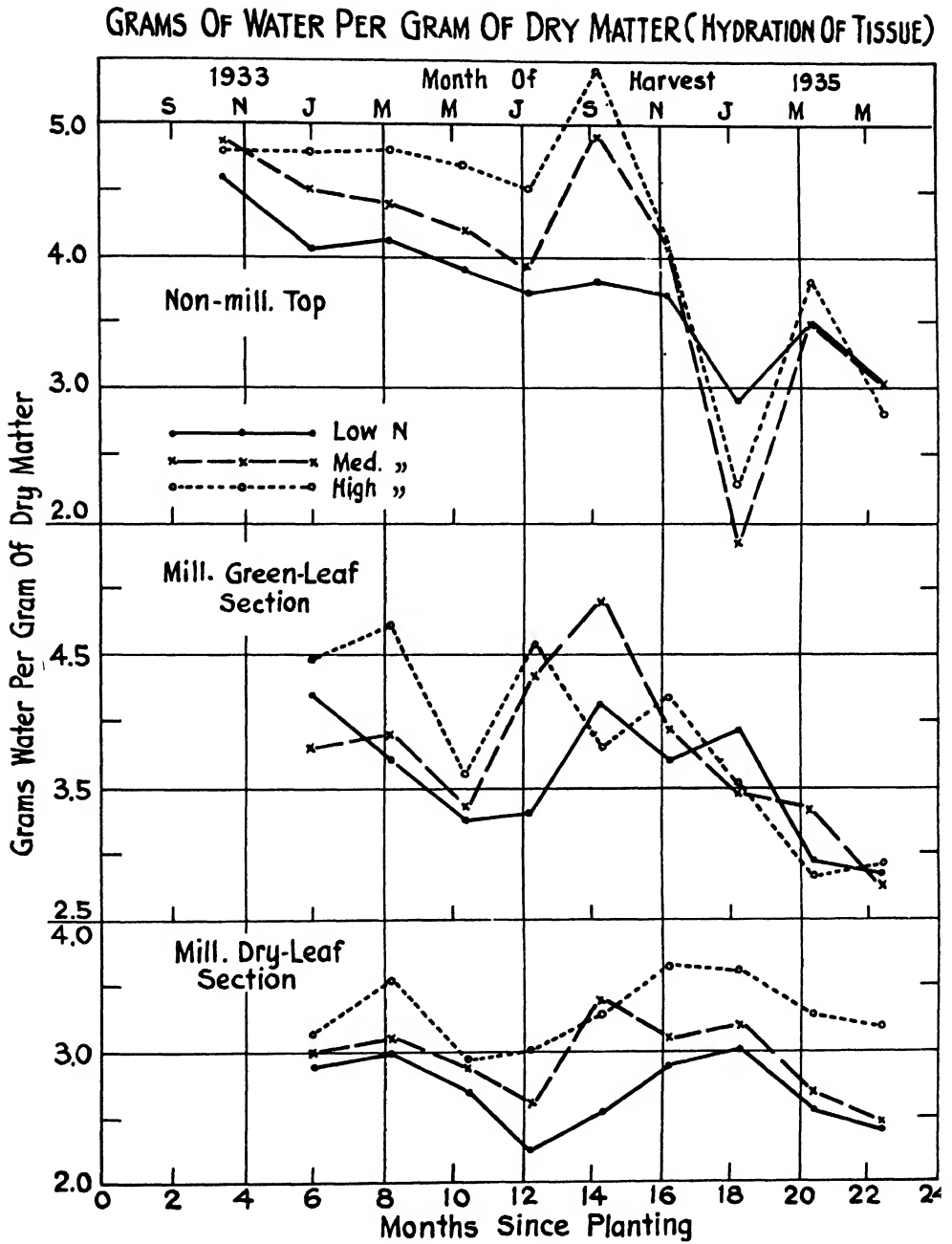


Fig. 6



ferences due to differences in water content, or are there actually less amounts of sugar in the highly fertilized plants even after eliminating water?" The first thing, then, we had to find out was, "Is there any more water in the high or the medium series than in the low?"

#### WATER CONTENT OF PLANTS (FIG. 6)

No doubt most of us have observed that well-fertilized plants look more succulent than the poorly fertilized ones. In other words, from common observation we might say that there will be more water in the plants receiving more nitrogen. Such is actually the case. From our data on the grams of water per each gram of dry matter (or pounds of water per pound of dry matter) in the plants of the three series, it is quite clear that as long as fertilization was continued, that is, up to the fourteenth month, the amount of water was always greater, the greater the amount of nitrogen applied. It is extremely significant that this holds not only for the leafy top but also for the millable green-leaf and the millable dry-leaf sections. After the stopping of fertilization, all three treatments become more or less alike in the recently grown top and the millable green-leaf sections but the differences accumulated in the past growth still continued to show in the dry-leaf section.

If we study the data a little more we discover that whereas in the young cane the top and the green-leaf parts were much more succulent than the dry-leaf, there was little or no difference between the various sections in eighteen or twenty month-old cane (i.e., months after the last dose of N was applied). We further discovered that while maintaining their respective differences in succulence, all the series showed very marked seasonal influences. For the dry-leaf sections, we find that the water content was the least in April, May, and June and that it reached the maximum around November and December.

There is considerable speculation but little agreement as to the cause of this difference in water content. We shall not discuss here the various arguments put forth but, instead, we shall just mention that both the treatment and the seasonal differences appear to be markedly correlated in our case with the amount of salts (potassium salts, phosphate salts, etc.) in the juice. This has led us to make the suggestion that the greater amount of salts present might actually be the cause of greater succulence. We hope to be able to find something more definite about this in our future studies.

#### SUCROSE CONTENT (FIG. 7)

We studied the sucrose content of cane in two ways: (1) by crushing the entire lot of cane through a "Cuba" mill and analyzing the juice, (2) by determining the sucrose in samples of cane tissue from which all water had been removed by heating in an oven. We found differences in sucrose not only between parts of plants but also between treatments and between seasons. The treatment differences were what we might have expected—namely, lower sucrose content from higher fertilization. However, even more striking were the seasonal differences—the best juice being obtained around May and June, and the poorest around November.

Seeing that we found differences in water content, we would be justified, if we did not examine the data very carefully, to draw the conclusion that differences in

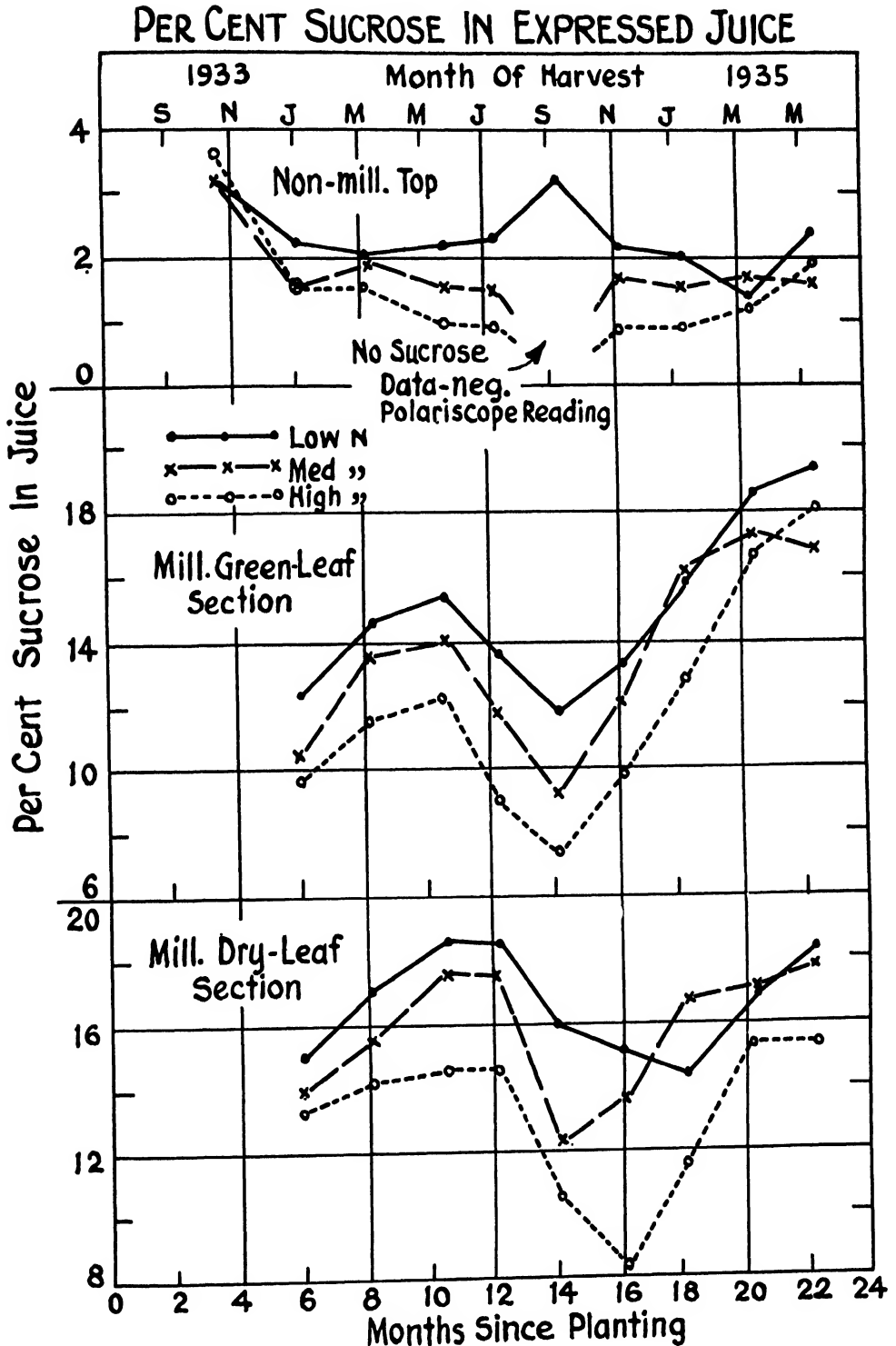


Fig. 7

sucrose content in the three series were purely the dilutive effect of water. In fact a strict mathematical analysis shows this to be largely the case. We say largely because on closer examination we find that the differences in sucrose content were too great to be explained by the differences in water content alone. In fact we found that the amount of sucrose per pound of water-free tissue was also less with increasing nitrogen fertilization. We are then led to the conclusion that as we apply more nitrogen the juice becomes poorer because of two reasons: first, the greater amount of water in the plant has a big diluting effect, and second, for some reason, there is actually less sucrose stored. We may now ask, "What may that reason be?"

If there is less sucrose stored, is there anything that is stored in greater abundance in the high than in the low series? On detailed examination of our other data we discovered such to be the case. As we applied more nitrogen we got more glucose in the plants. Furthermore, there appeared to be a clear case of antagonism between glucose and sucrose. When one is high the other is low and vice versa. Even more significant is the fact that if we added glucose and sucrose together then the sum total of these sugars was about the same in the three series in the water-free plant tissue. In other words, the plants in the high series were not necessarily less capable of making sugars but they were inclined to store the sugars manufactured by the leaves in the form of glucose rather than in the form of sucrose. If we were producing both glucose and sucrose in our factories, then we would have little complaint about the poor quality of juice as a result of heavy fertilization.

Various theories may be brought forward to explain the above results. However, we incline to the view that the different water content of the three series might be the actual factor involved. As we picture it, when the amount of water per pound of cane tissue is high, then a relatively large proportion of the sugars made by the leaves will be stored as glucose. Thus in the summer months when the plant normally has a large amount of water and is growing rapidly, glucose will be found in abundance in the juice. In the winter months, on the other hand, the water content of the plant is low and so is the glucose content, while the amount of sucrose is high. If we follow along the same line of reasoning, we should further find that as the young joints which at first contain a large amount of glucose become mature and lose a part of their water, there should be an increase in the sucrose at the expense of glucose. It will be readily seen that the simple conception that we have put forward is in harmony with many of our observations and cultural practices. Let us compare, for instance, the quality of the millable green-leaf and the dry-leaf sections. The former has less sucrose but more glucose than the latter but the sum total of these two sugars is practically the same in both the sections. We would, therefore, be justified in thinking that in passing to the dry-leaf section the green-leaf section lost some of its water and as a result some of the glucose had been converted into sucrose—the total of the sugars remaining the same. Take again the practice on irrigated plantations, of "drying off" before harvest; we would expect, if our theory were right, to find not only an increase in the concentration of sucrose as a result of loss of water from the cane but in addition we should find some change of glucose into sucrose.

We should remind our readers at this point that the conceptions here advanced though partly grounded on experimental evidence of workers abroad, are still far

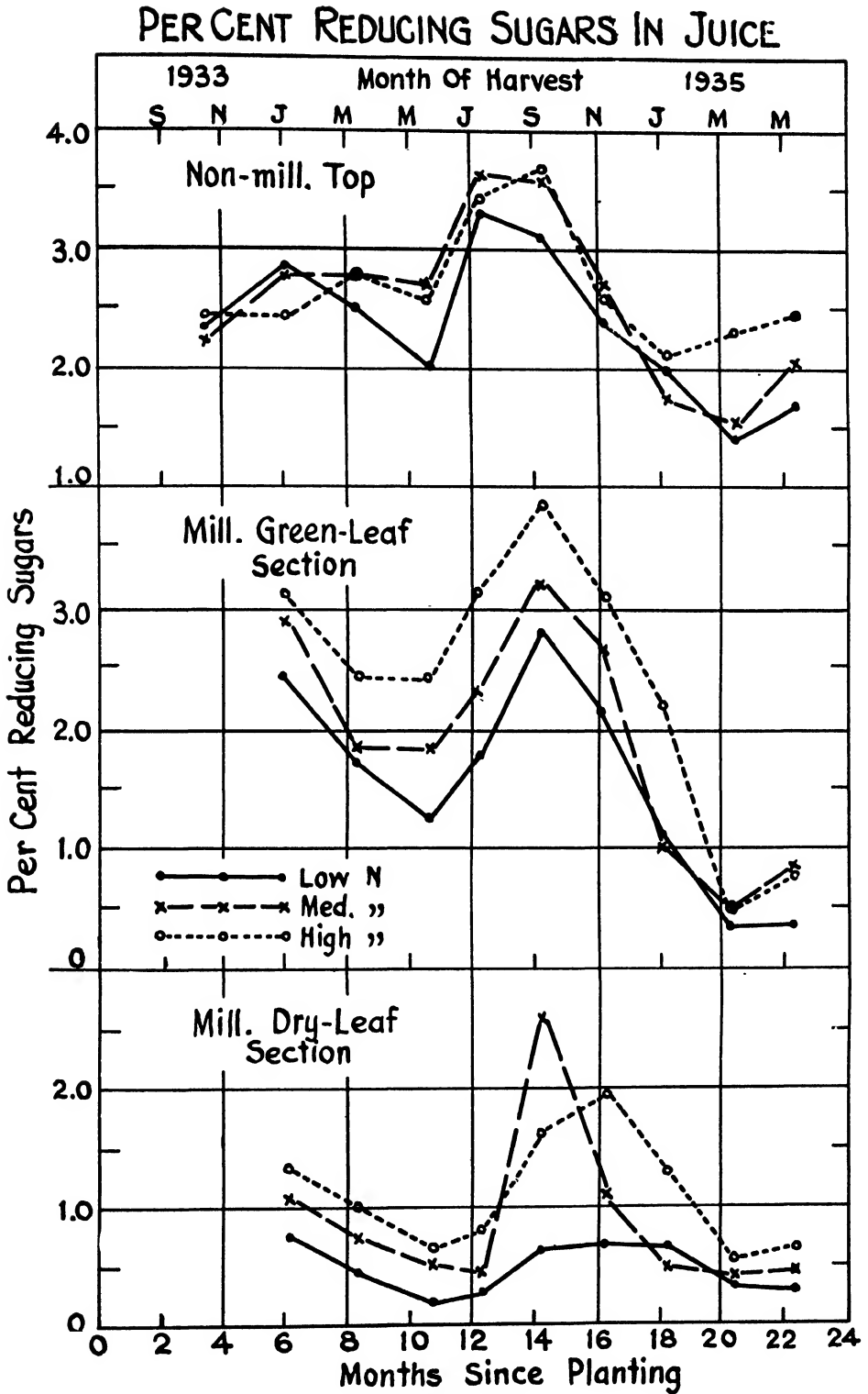


Fig. 8

from being entirely confirmed. We have planned future experiments from which we hope to learn more about the rôle of water in the cane plant.

Before leaving this topic, we should make it clear that if the water content of the tissue is the governing factor then anything that influences this will also influence the sucrose content. Thus cutting off the nitrogen supply, cool weather, cutting off the irrigation—all will have a similar effect on cane juice because all will decrease the water content.

### GLUCOSE (FIG. 8)

As we have pointed out in the last topic, glucose and sucrose seemed to be opposed to each other. The high-N series had the lowest concentration of sucrose but the highest of glucose. In the months of April to June, the juice of all series had generally the maximum concentration of sucrose but the minimum concentration of glucose. Of even greater interest is the general similarity between the glucose curves and the cane growth curves. We note that the period of maximum elongation (around September 1934) coincided with the period of maximum glucose, and the period of minimum elongation (around May) with that of minimum glucose. Similar relationship is also observed between the glucose content and the seasonal variations in the water content of the tissue. There is no doubt that these facts are at the bottom of our plantation practice of considering glucose content of juice as an index of maturity.

When we study the glucose curves we must take note of the fact that soon after our nitrogen fertilization was stopped, the glucose content of the recently developed cane, i.e., the millable green-leaf section, began to decrease steadily in all the series and simultaneously the water content also decreased while the content of sucrose went up. This is in agreement with our observations that in the old cane, which has received no fertilization for months, there is little difference between the quality of the dry-leaf and the green-leaf sections.

#### *Other substances of the basic nature of the sugars:*

We have also determined other substances that are chemically related to the sugars like glucose and sucrose but are of far greater complexity. Our purpose in so doing was to find out if the reasons for low sucrose content in the high-N series as compared with the low-N series were not partly due to the fact that in the high series there were relatively more of those complex substances. We have found no clear cut evidence yet that there is any such difference. This inquiry also will be pursued in more detail in our future work.

### NITROGEN CONTENT OF CANE

Since we were applying such varied amounts of nitrogen, we felt it would be of interest to know how much of it was being taken up by the plants in the three series. We have heard a great deal about luxury consumption in plants whereby they take up plant food regardless of immediate needs; this experiment should, then, be a good place to test this theory.

We report the results in two ways: (1) as the amount of nitrogen in each hundred grams of the water-free cane tissue and (2) as the amount of soluble nitrogen

# PER CENT TOTAL NITROGEN IN DRY MATTER

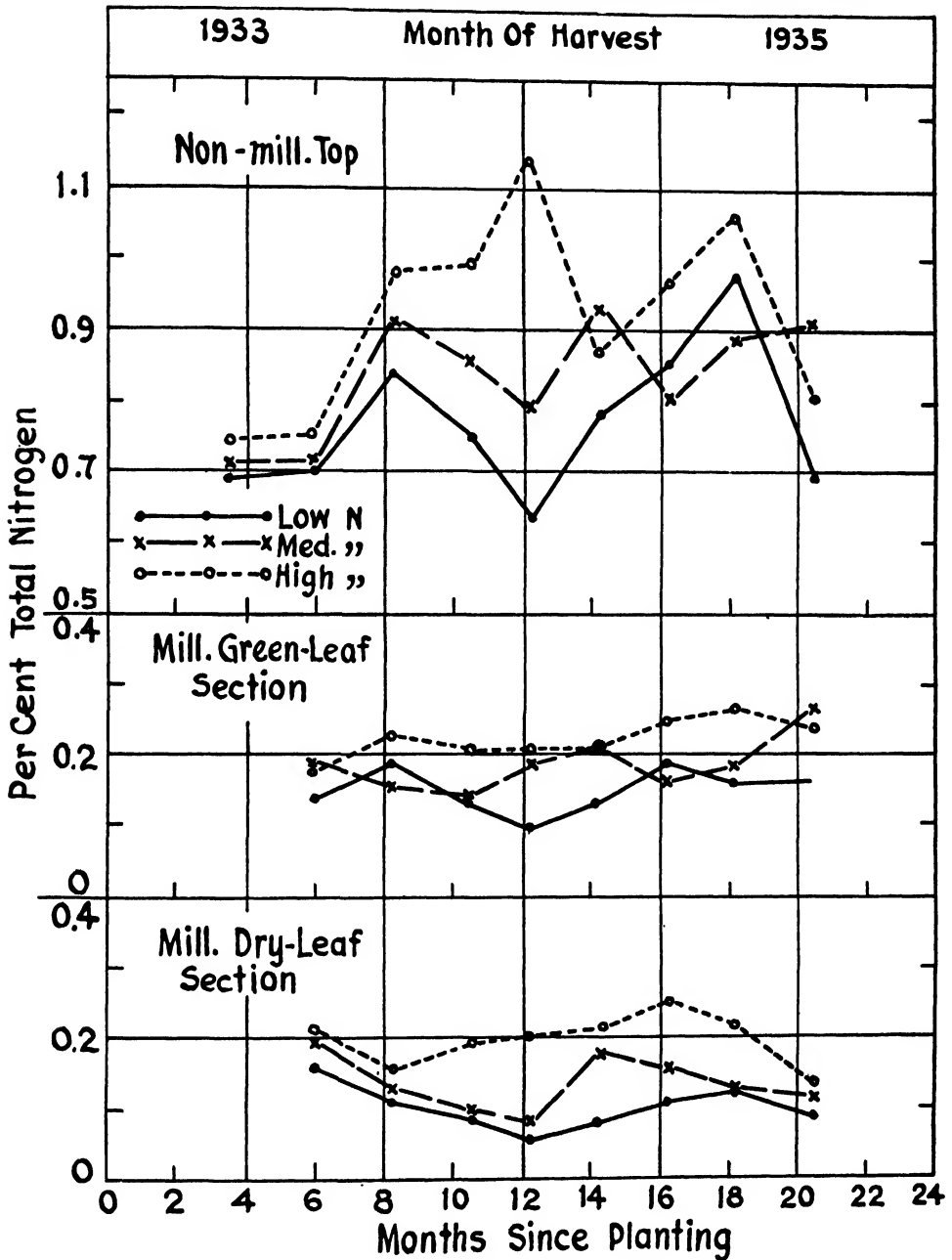


Fig. 9

in the same amount of dried tissue. The reason for the latter determination is that only a small part of all the nitrogen found in the cane plants is in a soluble form. From the chemical point of view we should expect that the soluble fraction will really be the active fraction just as the soluble fraction of the fertilizers we put in the soil is generally regarded as the growth-promoting fraction.

We find (Fig. 9) that as we applied more nitrogen, more was taken up by the plant and more was found in the tissue. We find the same thing with regard to the soluble fraction. However, we note that whereas the plants in the medium and the low series showed progressive decrease in the soluble fraction from the top down towards the dry-leaf section, in the high-nitrogen series all the sections had about the same concentration. From this we will be justified in concluding that in the high series there was such an excess of soluble nitrogen that all parts of the plants were equally gorged with it.

We have now calculated the total pounds of N found in the top, the green-leaf and the dry-leaf sections of the mother and daughter stalks (we have seen before that these stalks make up 95 to 100 per cent of the total cane weight), and have expressed this amount on an acre basis (Fig. 10). We find that as long as the application of nitrogen was continued, i.e., up to the fourteenth month, the high series was taking up nitrogen at an even rate, the medium series almost as evenly, but in the case of the low series the rate fell off with age. We should recall how growth and yield also fell off in this series immediately after fertilization was stopped.

Knowing the amount of N applied to the soil and knowing the amount found in the plants\* we can study the efficiency of uptake in the three series. In the lower part of Fig. 10 we have expressed the amount taken up as a per cent of the amount applied to the time of harvest.

Significantly, the uptake is 100 per cent or more in the low series showing that the plants must have actually drawn on the soil reserve. In the medium series, the uptake is about 75 per cent and only about 40 per cent in the high series. Evidently there is a limit to the plant's capacity to take up the applied nitrogen. If we now multiply the per cent uptake by the pounds applied, we find that the high series took up only about 260 pounds as against 200 pounds by the medium series and 140 pounds by the low series. The amounts found in the plants, therefore, give a much better idea of the relative yields of cane to be expected than the actual amounts applied to the soil.

At this point we venture to raise a question—"If we recover, say, about 80 per cent with an application of 266 pounds of N and fully 100 per cent or more with an application of 133 pounds, should we not find it profitable to apply a little less than 266 pounds, rather than an amount greater than this?"

We do no more than ask the question because we realize that the efficiency of utilization varies from year to year and from place to place. The man on the spot is, after all, the one best suited to answer the question for his locality and for his variety of cane.

\* We should mention that as we did not analyze the roots and the dry trash, our nitrogen balance is not exactly right but undoubtedly it is very close to being so as neither roots nor dry trash have much nitrogen or make up much weight.

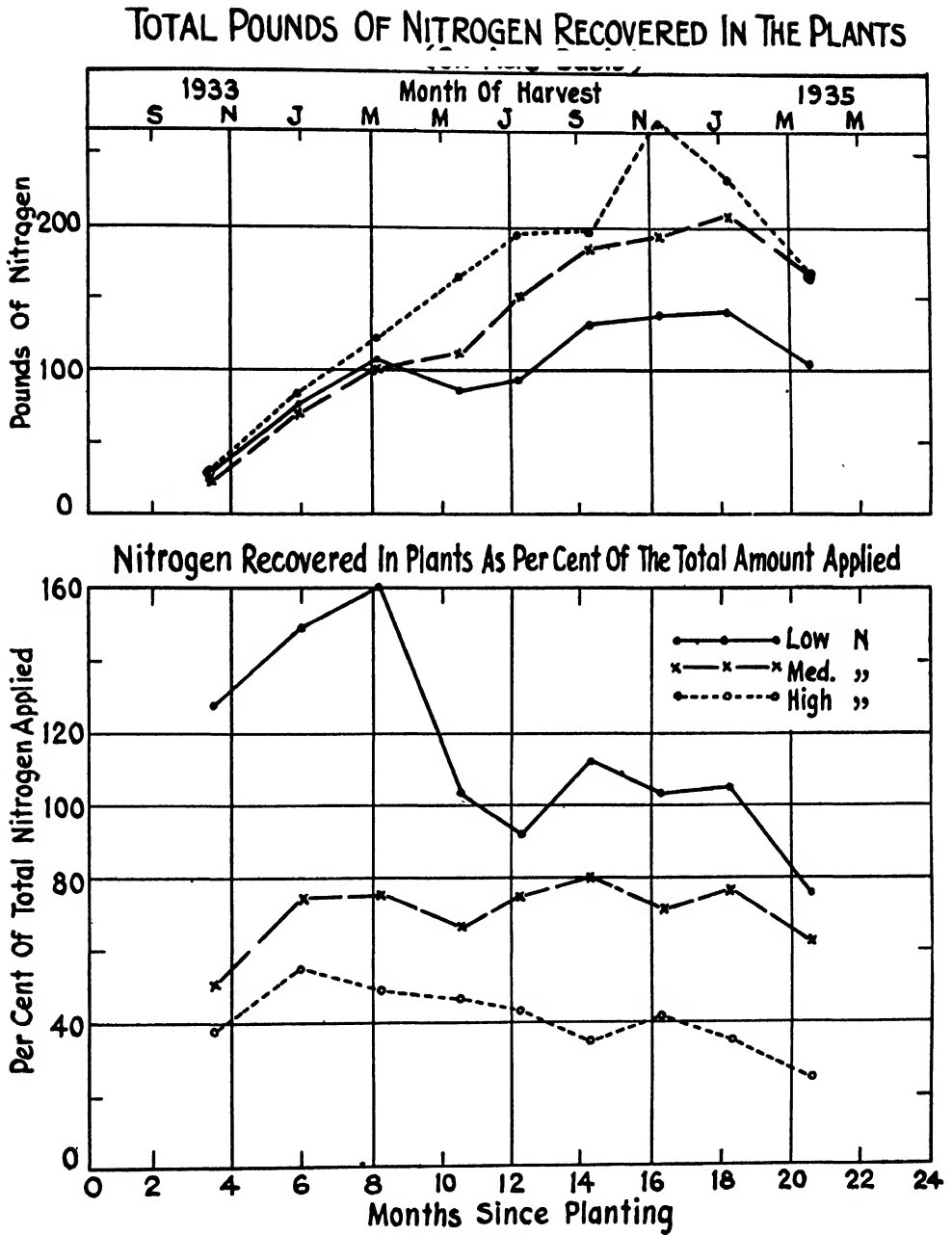


Fig. 10



## ELECTRICAL CONDUCTIVITY—OR THE AMOUNT OF ELECTRICITY THAT A SAMPLE OF JUICE CAN CONDUCT

Those of us who have some familiarity with the chemical methods employed in the laboratory to determine the amount of various salts in the cane juice, such as muriate of potash, or ammonium sulfate, know that these determinations are rather arduous and time consuming. If we were not really very particular whether we knew the different kinds of salts that were present in the juice but contented ourselves with knowing the total amount of those salts, then we could dispense with the tedious chemical methods and employ one that is much simpler. Such a method consists in determining the current of electricity that passes through a solution such as cane juice. Investigators have found that an electric current passes with difficulty through pure water but if the water contains salts, such as muriate of potash, then the current flows more readily, the flow depending on the concentration of the salts.

We have made regular determinations of the electrical conductivity of juice and show the results in Fig. 11. We find that the top is many times richer in salts of the type mentioned than the green-leaf and the dry-leaf sections. We further note that after the application of nitrogen was stopped, the green-leaf and the dry-leaf sections tended to become alike with respect to salt concentration. Most interesting, however, are the data on the dry-leaf sections. Not only do we find that the higher the application of nitrogen, the higher the conductivity, but also there are definite seasonal rhythms very much like those which we found with respect to water content, sucrose and glucose. We therefore conclude that as we apply more nitrogen we increase the salt content of juice, but seasonal variations will predominate over treatment variations.

It will be recalled that we have already suggested that the differences in the water content might be really due to these differences in the salt content.

We should further observe that the conductivity of juice and sucrose content appear to be definitely related although in an opposite sense, i.e., when conductivity is high, sucrose content is low and vice versa. This is not surprising in view of the already discovered fact that the water content is related to sucrose content and also to conductivity.

The question we might ask now is, "What causes the seasonal differences in conductivity?" We do not know the answer for certain. However, much work is being conducted abroad on this topic and we hope that a definite solution will be given for this problem before long.

## ACIDITY OR pH OF JUICE

We also determined the pH of juice. In general the pH is higher as we go from the top down the stalk. As higher acidity is denoted by a lower pH value and vice versa, we conclude that the leafy top is the most acid and the dry-leaf section the least. In this latter section there appeared to be a slight increase in acidity as the cane became older. Some plants actually do store acids and thereby increase in acidity; whether or not the sugar cane plant does that to any extent we do not know. We shall study this point more fully in the future.

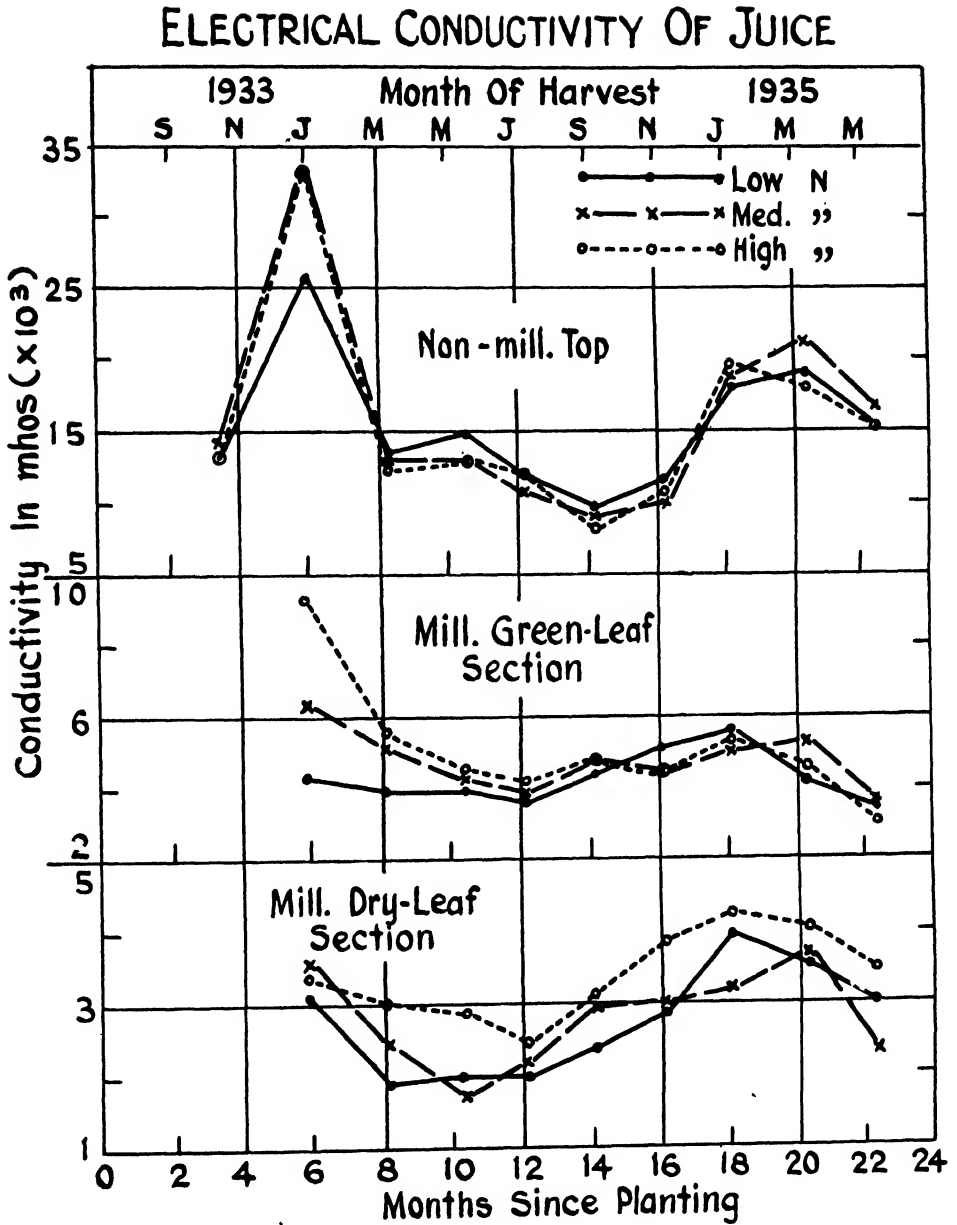


Fig. 11

## TOTAL POUNDS OF SUGAR

We have seen that the cane tonnage is highest in the medium series from the eighteenth month on because of increasing mortality in the high series. If we now obtain from the data on tonnage and sucrose content the actual pounds of sugar in each line, we find that it is the highest in the medium series. The high series, on the other hand, is only slightly better than the low. Evidently what the low-N series lacks in tonnage it makes up in quality. The medium series is really the golden mean, where high tonnage is combined with fairly high quality. There is little novelty in this deduction for we know as a result of many field experiments that an amount of N around 266 pounds does give us the highest yield of sugar in the well-irrigated fields of Oahu. It is gratifying, however, to see our previous experience supported by a different approach.

## THE WHOLE PICTURE

In discussing the various topics, we have referred to the various inter-relationships; here we bring them together in a single picture. In Fig. 12 we show the data on nitrogen in the tissue, electrical conductivity of juice, water content of the tissue, and the concentration of sucrose and glucose in the juice of the dry-leaf section. We find general similarities in all the curves not only with regard to the differences between the series but also between seasons. We would like to draw particular attention to the data on the harvest in September (age fourteen months) for we feel that here may be a case where an apparent exception may prove the rule. We find that the medium series shows a sudden deviation, and that this deviation is present in all of the curves. Surely this could not be a mere coincidence; instead we feel it supports our contention that all the juice characteristics shown in Fig. 12 are in fact related.

It would appear here as if in the dry-leaf section the complexities stand partly unravelled, thus affording us a view of the many inter-relations through which nitrogen affects the quality of juice. The sequence of events, as we see it, is as follows—"Application of nitrogen increases the salt uptake as indicated by the electrical conductivity of juice. The greater salt content causes greater succulence, i.e., greater water content. The greater amount of water present has first a diluting effect on the juice and secondly, it may even determine as to whether the sugars formed by the leaves are to be stored as sucrose or as glucose." This picture may not be entirely correct; later experiments may modify our views as to which is the first cause and which the second, but we feel that the one presented may be taken at least as a working hypothesis.

## SUMMARY

We can sum up the high points of our experiment thus:

- (1) The higher the application of nitrogen, the greater will be the tiller production, the greater the number of joints formed and the higher the rate of cane growth.
- (2) The higher the application of nitrogen the greater the tonnage of cane. However, with excessive nitrogen, mortality of cane may be so great as to produce less cane than with a moderately high application.

# INTER-RELATION OF VARIOUS CONSTITUENTS IN THE MILLABLE DRY-LEAF SECTION

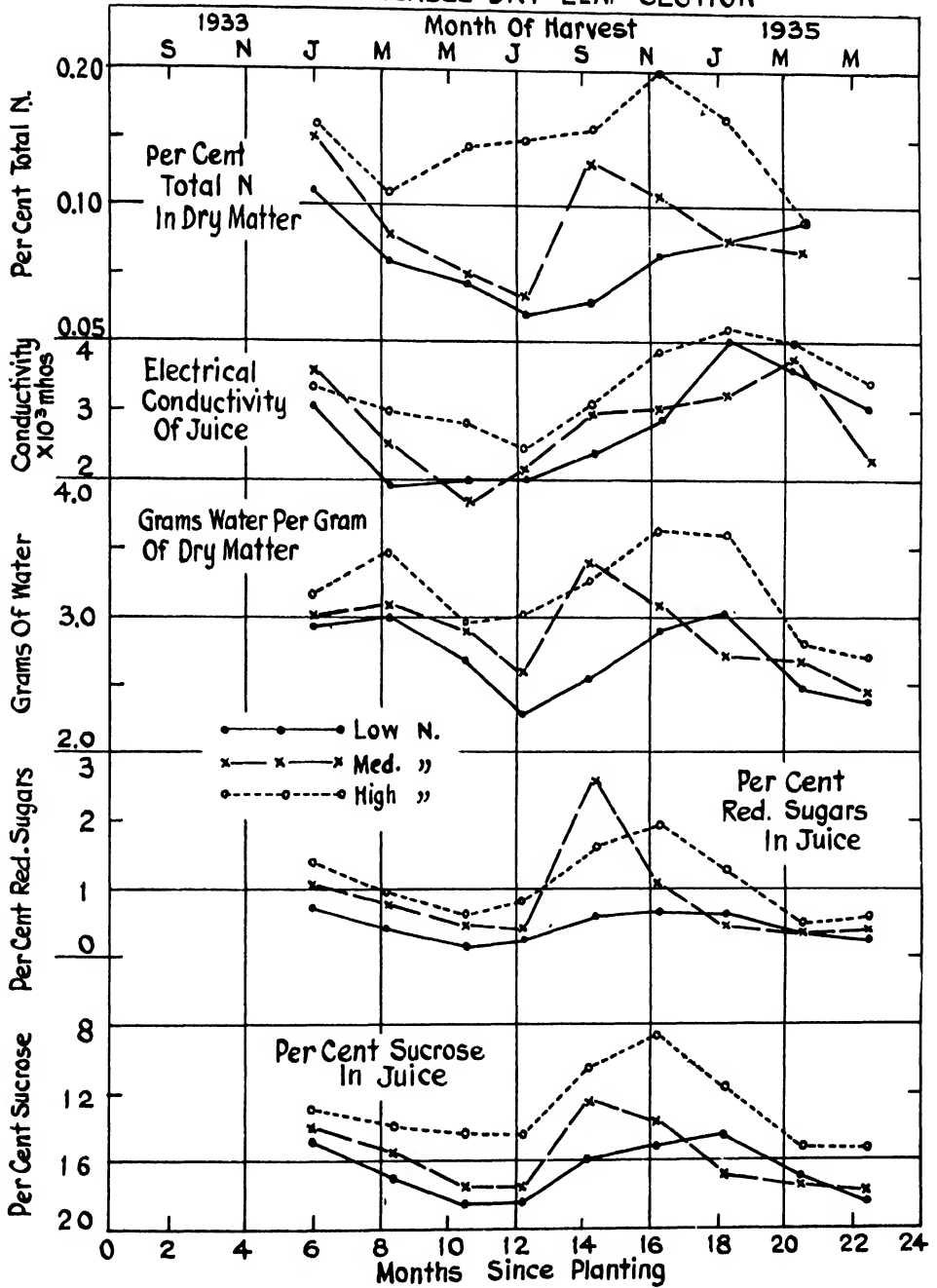


Fig. 12

(3) The more nitrogen applied, the greater the succulence, i.e., the water content, of plants. We suggest that the increased water content has two important functions: (a) it dilutes the juice, resulting in a lower concentration of sucrose with higher amounts of nitrogen, and (b) it probably determines whether or not the sugars manufactured by the leaves will be stored mainly as sucrose or a fair proportion of the sugars will be in the form of glucose.

(4) The greater the application of nitrogen, the poorer will be the concentration of sucrose in juice as well as in the water-free tissue, while the concentration of glucose is just the reverse.

(5) The highest amount of sucrose is obtained from moderately high applications of nitrogen. An excessive application of nitrogen, as in our high series, produces such poor juice as to largely offset the advantages of increased tonnage.

(6) The greater the application of nitrogen, the greater the nitrogen content of the tissue. The efficiency of uptake of nitrogen decreases as the amount applied is increased. With low applications fully 100 per cent may be recovered in the plants, while with excessive applications as in our high series only about 40 per cent is found in the plants.

(7) The electrical conductivity of juice (which is a measure of dissolved salts such as muriate of potash) increases with increasing applications of nitrogen, indicating that nitrogen stimulates the absorption of salts. We suggest that the increased salt content might be the reason for the increased succulence of plants receiving greater amounts of nitrogen. Electrical conductivity and sucrose content of juice appear to be oppositely related to each other.

(8) Although not clear in the details, yet we can discern the broad outlines of a rather simple picture. Increasing applications of nitrogen appear first to increase the salt absorption by the plants, the increased salt content probably causing greater water content or succulence of tissue. The greater water content has a diluting effect on juice resulting in a lower concentration of sucrose. This increased water content may even favor the storage of sugars such as glucose at the expense of sucrose.

There are many other points of interest which have come out in this study, points that largely confirm our past observations and our existing cultural practices. Many points need yet to be clarified before we can feel entirely at ease regarding the possible influences of nitrogen fertilization on quality. This study has given us many good leads and it is our purpose to follow these carefully.

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# Biological Control of the Sugar Cane Leafhopper in Hawaii

By O. H. SWEZEY

## CONTENTS

	Page
Foreword .....	58
Early History of the Leafhopper in Hawaii.....	58
Plans for Introduction of Parasites.....	62
Koebele's Work in Ohio.....	63
The Leafhopper Determined as a Queensland Species.....	64
Organization of Division of Entomology.....	65
The Perkins and Koebele Expedition to Australia.....	66
Introduced Parasites: .....	67
<i>Paranagrus optabilis</i> .....	67
<i>Paranagrus perforator</i> .....	72
<i>Anagrus frequens</i> .....	73
<i>Ootetrastichus beatus</i> .....	73
<i>Ootetrastichus formosanus</i> .....	74
<i>Haplogonatopus vitiensis</i> .....	76
<i>Pseudogonatopus hospes</i> .....	76
<i>Cyrtorhinus mundulus</i> .....	79
Natural Enemies Which Were Already Present in Hawaii:.....	81
<i>Echthrodelpax fairchildii</i> .....	81
<i>Coelophora inaequalis</i> .....	84
<i>Conocephalus saltator</i> .....	85
<i>Zelus renardii</i> .....	88
<i>Reduviolus capsiformis</i> .....	89
<i>Reduviolus blackburni</i> .....	89
<i>Triphleps persequens</i> .....	91
<i>Physopleurella mundulus</i> .....	91
<i>Oechalia grisea</i> .....	91
<i>Chelisoches morio</i> .....	91
<i>Euborellia annulipes</i> .....	91
<i>Chrysopa microphya</i> .....	93
<i>Anomalochrysa deceptor</i> .....	94
<i>Anomalochrysa gayi</i> .....	94
<i>Anomalochrysa raphidioides</i> .....	94
<i>Anomalochrysa proteus</i> .....	94
<i>Nesomimesa hawaiiensis</i> .....	94
<i>Pipunculus juvator</i> .....	95
<i>Pipunculus terryi</i> .....	95
<i>Pipunculus hawaiiensis</i> .....	95
Spiders .....	96
Enemies of Spiders.....	97
Fungus Diseases of the Leafhopper.....	98
Literature Cited and Bibliography .....	98
Appendix A .....	100

## FOREWORD

In the lengthy campaign for the biological control of the sugar cane leafhopper in Hawaii, much has been published from time to time concerning the occurrence of the leafhopper in Hawaiian cane fields, studies of its life history, etc., and separate accounts of the introduction of the various parasites. Now that the leafhopper has been under satisfactory control for many years, and there is every reason to believe that this situation will continue, it is well that a connected account be given of the history of the occurrence of this pest in Hawaii, and the history of the introduction of the various parasites with details of their methods of attack and relative importance in the ultimate control of so serious a pest, which in 1903-04 threatened to ruin the sugar industry in Hawaii. The present paper is an effort to assemble briefly what has been accomplished, and how, with numerous references to literature where fuller details have been published in due course. The illustrations have often appeared in previous publications by the Experiment Station, H. S. P. A.; in bulletins from the Entomology Department; in *The Hawaiian Planters' Record*; and in the volume on "Insects and Other Invertebrates of Hawaiian Sugar Cane Fields," by F. X. Williams.

## EARLY HISTORY OF THE LEAFHOPPER IN HAWAII

The sugar cane leafhopper\* (8, p. 179) in Hawaii was a pest of foreign origin. Its first observance in Hawaii was made by Dr. R. C. L. Perkins when taken at light in his room at Waialua, Oahu, in the latter half of 1900. It was recognized at the time as an immigrant insect, not previously seen, but its habits or importance were not known until about a year later. Dr. Perkins had spent a number of years in collecting insects on the various Islands, beginning in 1890, and although he had collected many species of endemic leafhoppers, he had not previously seen this one, which, while related to the native leafhoppers, yet was distinctly different. Later, when its importance became evident, Dr. Perkins concluded that it must have been quite a recent introduction, or he would have noticed it sooner. He considered that it must have been introduced two or three years prior to 1900.

The first record of the leafhopper attacking sugar cane is found in the annual report of Albert Koebele for the year 1901 (14, p. 21). In this report he mentions E. G. Clarke, then Agriculturist at the Experiment Station, H. S. P. A., as reporting the appearance of the leafhopper on sugar cane at the Experiment Station some twelve months previously. This would have been either late in 1900 or early in 1901, not long after, or about the time when Dr. Perkins took specimens coming to light at Waialua. It must have been present in the cane fields at Waialua at that time, without its presence having yet become known. In Mr. Koebele's report, Demarara and Rose Bamboo were the varieties of cane affected. He described the characteristic appearance of cane when badly attacked, viz., the black and dirty leaves caused by the excreted honeydew in which sooty mold flourishes, and the red midribs

\* *Perkinsiella saccharicida* Kirkaldy. (The numbers in parentheses refer to literature cited.)

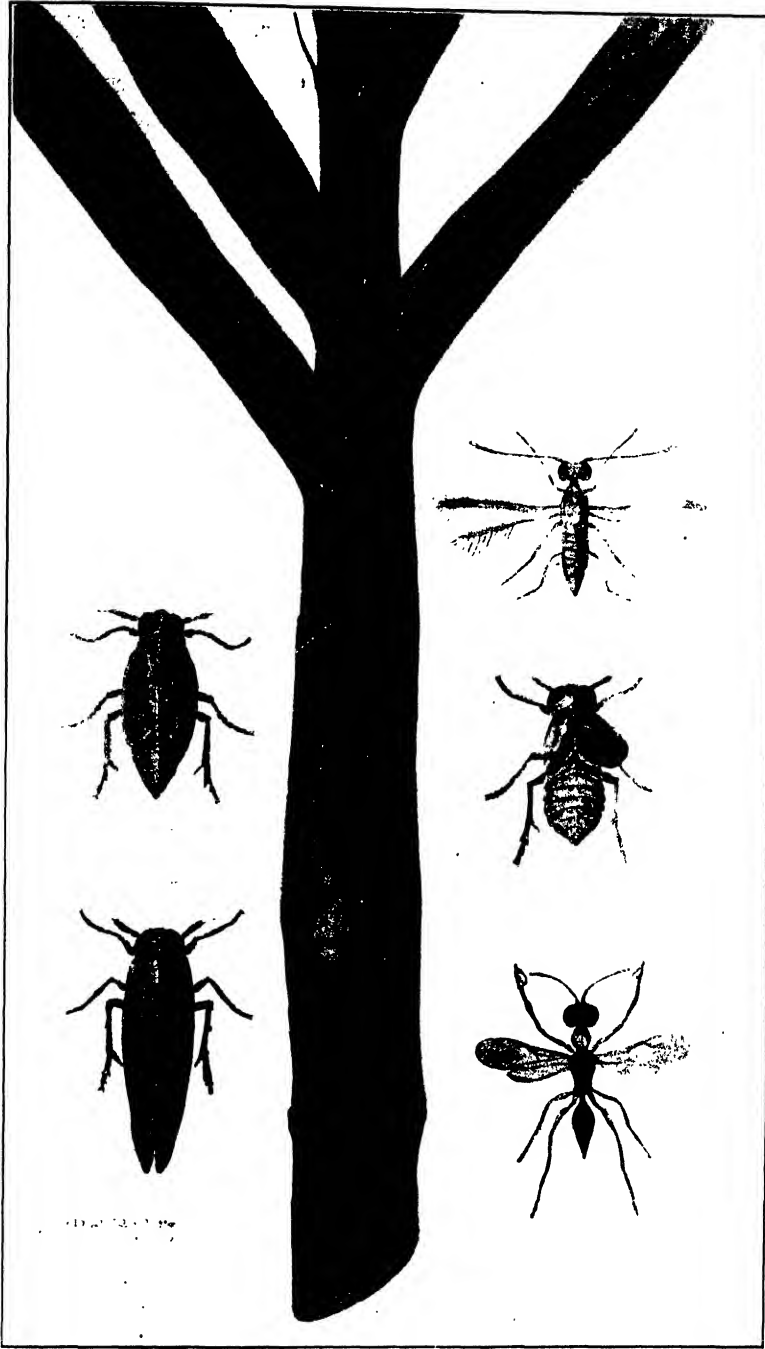


Plate 1. Sugar cane leafhopper (*Perkinsiella saccharicida*).

Center: Sugar cane stalk infested with leafhoppers; at left, adult leafhoppers, long-winged and short-winged, enlarged; upper right, egg parasite (*Paranagrus optabilis*) greatly enlarged; right intermediate, young leafhopper showing black larval sac of Fairchild's parasite, considerably enlarged; lower right, Fairchild's parasite (*Echthrodelpfax fairchildii*), adult female, considerably enlarged.



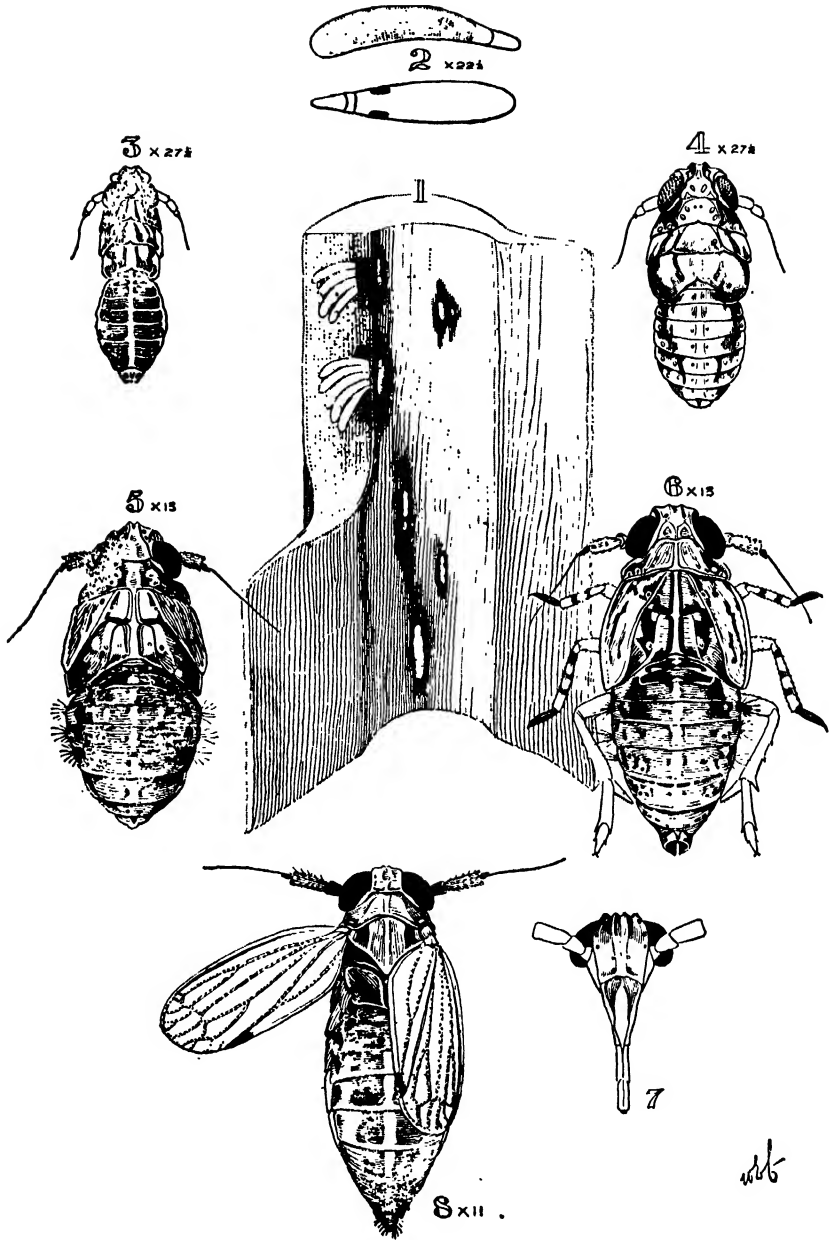


Plate 2. Sugar cane leafhopper (*Perkinsiella saccharicida*).

1. Eggs in sugar cane midrib, those at the side being shown somewhat in profile and in section.
2. Eggs isolated, highly enlarged.
3. Nymph or young of first instar.
4. Young, second instar.
5. Young, third instar.
6. Young, fourth instar.
7. Face of the same.
8. Adult female, short-winged form.

of the leaves caused by the fermentation taking place at the injuries where the leafhopper eggs were inserted in large numbers.

Attention having been brought to these conditions, the presence of the leafhopper was soon made known on all plantations of Oahu.

Dr. Perkins in a paper, "Notes on insects injurious to cane in the Hawaiian Islands" (31), stated that the leafhopper was ubiquitous on Oahu in 1902 and also known on Kauai, and known only from these two Islands. It was already recognized as a serious cane pest, the destructiveness of which threatened to exceed that of the cane borer beetle. Dr. Perkins advised that care should be taken to prevent its spread to the other Islands, if it were not already established there. However, it must have been already established for some time on these Islands, for D. L. Van Dine (57, p. 8) has reported that his attention was first called to it on Maui by Hon. H. P. Baldwin on cane at Puunene, August 12, 1902; and early in 1903 it was reported from some of the sugar plantations on Hawaii, where it had already become conspicuously injurious to the cane.

The manager of Paauhau Sugar Plantation Company on Hawaii reported its first appearance about the end of February and again on May 28, 1903, he reported it "on every acre of the plantation."

Early in June 1903, Mr. Van Dine (57, p. 8), the entomologist at the Hawaii Agricultural Experiment Station in Honolulu, visited Paauhau after having spent two weeks studying the habits of the leafhopper in the Kohala cane fields. He pronounced the leafhopper infestation worse there than in Kohala. Several plantation managers of the Hilo and Hamakua districts also visited Paauhau to examine the leafhopper injuries. All pronounced this the most severely infested plantation, although the leafhopper was present throughout the plantations on the windward coast of Hawaii, but not yet injuring the cane as at Paauhau, where some fields of cane were severely checked in growth. Yellow Caledonia was the principal cane grown in the Hilo district and it was injured less than the Rose Bamboo cane, which in turn was more severely injured at Paauhau than was the Yellow Caledonia cane grown in the same place. At this time the plantations at Ookala and Hamakua were infested about the same as at Paauhau.

Dr. Perkins visited Paauhau, Honokaa and Kukuihaele, June 17-24, 1903, and made studies of leafhopper conditions and of the natural enemies which were already increasing in the fields and helping to check the leafhopper. The results of his observations were published in a bulletin (32) soon after.

The most severely injured plantation on the Island was the Hawaiian Agricultural Company at Pahala, where the Yellow Bamboo cane was the variety chiefly grown, and it proved to be more susceptible to leafhopper attack than any other variety. The damage was so extensive here that whole fields of great area were practically killed outright, and the plantation, which had a sugar crop of 18,888 tons in 1903, was reduced to crops of 1,620 tons in 1905 and 826 tons in 1906. These two latter crops were from cane that was growing and was practically ruined in the worst leafhopper years, representing a loss of over a million dollars each year.

In 1904, replacement of Yellow Bamboo was begun by planting the Yellow Caledonia variety as extensively as possible, and for a few years this variety in-

creased by about 2000 acres annually, resulting in bringing the Hawaiian Agricultural Company back to a sugar crop of 11,630 tons in 1907 and about that same amount for several years after, but not up to its pre-leafhopper yield for about 7 years. As previously stated, the Yellow Caledonia variety was not so severely injured by leafhopper attack, even though the pest was present in enormous numbers, so that when this plantation became largely planted to the Yellow Caledonia cane not much loss was involved.

At the time of maximum leafhopper damage, the total annual output of sugar from all plantations dropped from 437,991 tons to 367,475 tons, a difference of 70,516 tons, equivalent to a loss of over five million dollars. This occurred within two years of the time that the leafhopper was first known on sugar cane, which shows how quickly it rose to prominence. Of course, as stated by Dr. Perkins above, the leafhopper must have been present in the cane fields for several years without being noticed. There were no entomologists making regular visits to the plantations in those days, or its presence would have been known sooner. Apparently as soon as attention was called to it, it was already to be found in all districts.

Just how or when the leafhopper arrived in Hawaii can never be determined, but it is very evident that it came along with importations of cane cuttings for planting. Dr. Perkins (31, p. 595) related having examined a consignment of cane cuttings from Queensland containing eggs of a leafhopper, and that a few young leafhoppers were present, having hatched on the journey. (Needless to say, the package was destroyed.) It is quite likely that at some previous time infested cane cuttings were received and planted. The Yellow Caledonia and Rose Bamboo varieties were introduced from Queensland about 1880 (5), together with several other varieties which were planted in the Kau district of Hawaii. After growing there for several years these varieties were distributed to other districts. It is very probable that among the original consignment there were some canes containing leafhopper eggs, which hatched after planting, and some of the young survived to reach maturity on the new shoots of cane and succeeded in becoming established there. From this small beginning they had no doubt increased to such an extent that when cuttings were distributed elsewhere a few years later they all contained a few leafhopper eggs and in this way the pest became widely spread and would have had ample time to have already reached destructive abundance when found in 1902.

(For an account of the difficulty in getting the determination of this leafhopper, see page 64.)

#### PLANS FOR INTRODUCTION OF PARASITES

The Hawaiian Sugar Planters' Association had no entomological department at this time (1902). Mr. Koebele was government entomologist and had been introducing ladybeetles and parasites for scale insects, mealybugs, aphids, etc., since 1893, and in 1902, when it was realized that the leafhopper was a serious pest, was on an expedition to Mexico (May to December) studying the insects attacking *lantana* there and endeavoring to introduce some of them into Hawaii. The sugar planters were so much concerned that they wished Mr. Koebele recalled and a

start made on locating and introducing parasites for the leafhopper. The situation is expressed by Dr. Perkins, who was assisting Koebele by handling the consignments of insects sent to Honolulu, in a letter to Koebele dated October 24, 1902:

The Sugar Planters are now very much concerned about the leafhopper and some are shouting for your recall. I wrote my view of the matter to Mr. Tenney at his request. . . . I have taken all possible means to obtain some preliminary knowledge about this leafhopper, without which we should be acting blindly. To myself the matter appears very important. If the lantana business is not completed this year I think it could be continued at some other time, for both the ranchers and others are keen about its succeeding. I don't expect you now to get through until January, owing to the failure of the last lots, due to delayed arrival. . . . Of course, you will do what you yourself think best about returning for leafhopper. It really is serious, probably hundreds of thousands of dollars will be lost on the future crop and much more later on.

In Mr. Koebele's reply to this letter, dated November 23, 1902, from Cuernavaca, Mexico, he says:

I am very sorry about the increase of the sugar cane leafhopper and fear it will be another hard problem. Yet it is not like this lantana question, being only a simple matter of finding some efficient enemy or parasite. If any such exists it must be hunted up; if not, there is not much to be done, but I should think something can be found. As to the leafhopper, I should advise you to send specimens to Dr. Treub, Director of the Botanical Gardens, Buitenzorg, Java, and ask if they know the species. . . . The end of January ought to see me in Honolulu to start without delay in search of enemies for the cane leafhopper.

Again, in a letter of November 26, Mr. Koebele writes on the leafhopper problem:

On my return I shall not stay in Honolulu for any length of time, but shall start as soon as possible on that leafhopper business and I hope you will remain, while I am away on this matter. (This latter remark was because Dr. Perkins had made plans for a trip to England.)

Mr. Koebele had been seriously ill several times while in Mexico, and when he returned to his home in Alameda, California, in December, he was suffering from a bad attack of fever. He wrote December 26:

I am still unwell, not yet over my fever, but a rest may help me and I am only too glad to be out of Mexico and rid of the hardest work that I ever did.

Instead of returning to Honolulu as soon as he had planned, it was necessary for him to remain in Alameda for recovery. Hence, his plan for early return to Honolulu and a start on search of leafhopper enemies was not fulfilled. After having recovered from fever, some study was made of parasites of leafhoppers in California and Ohio.

#### KOEBELE'S WORK IN OHIO

Mr. Koebele went to Ohio on the advice of Dr. L. O. Howard, Chief of the U. S. Bureau of Entomology, to look up certain dryinid parasites which had been discovered by the writer (at that time a research student in Entomology at Ohio State University, Columbus, Ohio), working on certain leafhoppers in the fields of Ohio. An account of these parasites was printed in *The Ohio Naturalist*,

Vol. III, pp. 444-451, pls. 20-21, 1903. Upon Mr. Koebele's arrival in Ohio in August 1903, the writer worked with him a short time, showing him the situations where parasitized leafhoppers had been obtained, etc. Mr. Koebele worked the rest of the summer in Ohio, collecting and breeding leafhopper parasites for shipment to Honolulu. Besides those already known, he discovered several other kinds of parasites on leafhoppers. The parasites already known were Dryinidae: *Dryinus ormenidis* Ashm., living on the young of *Ormenis septentrionalis* Spin., and *Gonatopus bicolor* Ashm. (later described as a new species by Dr. Perkins under the name *Haplogonatopus americanus* [35, p. 40]), living on the young of *Liburnia lutulenta* Van D. Mr. Koebele discovered several other species of dryinids working on leafhoppers. He reared these parasites by the hundreds to the cocoon stage, in which condition they were sent to Honolulu. Cocoons of several species were sent, but none of them was ever known to have become established on the sugar cane leafhopper, although two species did attack the cane leafhopper and were reared to maturity in captivity and their offspring liberated in cane fields.

Other leafhopper parasites discovered were the egg parasite *Anagrus columbi* Perk., parasitic on *Liburnia* eggs; a minute stylopod of the genus *Elenchus*; and one instance of parasitism by a dipterous maggot of the genus *Pipunculus*.

Although no parasites became established in Hawaii from Mr. Koebele's studies in Ohio, the knowledge gained of the kinds of parasites that attack leafhoppers became of great value in later work with leafhopper parasites. What special kinds of parasites to look for was known when search was made in Queensland cane fields.

#### THE LEAFHOPPER DETERMINED AS A QUEENSLAND SPECIES

Considerable time elapsed before the identity of the cane leafhopper had been determined and it was ascertained from whence it came. From a study of the literature available in Honolulu at that time, Dr. Perkins stated in a report of October 23, 1902 (quoted in 32, p. 7) that he was certain it was not any of the species of leafhoppers known to attack cane in other countries. At the same time he called attention to the similarity in habits between the species in Hawaiian cane fields and the species in Javanese cane fields, *Dicranotropis vastatrix* Bred.:

Finally, after much correspondence with other countries, the matter was conclusively settled for me by Mr. Kirkaldy, who obtained from Germany cotypes of the Javanese insect described by Breddin and found it to be quite distinct from the Hawaiian one. Other authorities considered the Javanese insect and ours identical.

Meanwhile I was also corresponding with Australian entomologists in the hope of procuring specimens of a Queensland cane-infesting leafhopper for comparison with ours; but it was not till some six months after I began this correspondence that I had the great satisfaction of receiving from Mr. James Clark of Cairns, four specimens of this Queensland species, which proved to be the same as our own. Mr. Clark also informed me that this leaf-hopper had been known there for years, that it was their only species, that it did no noticeable damage and was probably kept in check by some efficient natural enemy.

As I have mentioned in former reports the fact that leaf-hopper was present on cane in Queensland was discovered by me when inspecting some seed-cane imported from that country, the said seed-cane containing numerous eggs of a leaf-hopper, while a few very young insects were also present. These not being at a stage of development when their identity with our own species could be decided, it was only on receipt of Mr. Clark's specimens that this was finally settled.

It was found that the species of the Queensland leafhopper had not been determined. Already Kirkaldy had described the leafhopper in Hawaiian cane fields as a new species for which he also erected a new genus—*Perkinsiella saccharicida*, being the name of this leafhopper as published in *The Entomologist*, Vol. 36, p. 179, July 1903. (Many more species of the genus *Perkinsiella* were discovered later, occurring on sugar cane in various regions. For list, see Appendix A.)

#### ORGANIZATION OF DIVISION OF ENTOMOLOGY

At the time when the leafhopper came into prominence as a sugar cane pest in Hawaii, there was no provision for work in entomology at the Experiment Station of the Hawaiian Sugar Planters' Association. Mr. Koebele was the Superintendent of Entomology, working under the Board of Commissioners of Agriculture and Forestry, and he had as assistant Dr. Perkins, who had spent most of the time since his arrival in the Islands in 1892 in collecting and in working on material for the *Fauna Hawaiiensis*, which in the main deals with insects. At the Hawaii Agricultural Experiment Station, Mr. Van Dine was staff Entomologist.

Early in 1903 the work of the Board of Agriculture and Forestry was reorganized in order to give more attention to the inspection of imported vegetable matter, and prevent as far as possible any further introduction of insect pests by way of the port of Honolulu. As Mr. Koebele was away most of the time in search of beneficial insects, Dr. Perkins was really in charge of this work. Late in the same year, Messrs. G. W. Kirkaldy and F. W. Terry were added to the staff to assist in inspection work. All of this work was carried on by an arrangement with and mostly at the expense of the Hawaiian Sugar Planters' Association, under which agreement much of the time was to be devoted to the study of sugar cane pests and the search for and introduction of beneficial insects to combat such pests. Of course the leafhopper was of most importance in this respect. Mr. Van Dine, of the Hawaii Experiment Station, had also given some attention to the study of the pest.

In 1904 the pest had assumed such importance that it became apparent that the Hawaiian Sugar Planters' Association should conduct the campaign against it independent of other institutions. Accordingly plans were started for organizing a Division of Entomology at the Experiment Station, H. S. P. A. When this organization was completed in the middle of the year, the entomologists of the Board of Agriculture were taken over by the newly formed Division of Entomology, the staff of which was made up as follows: R. C. L. Perkins, Director; Albert Koebele, Consulting Entomologist; G. W. Kirkaldy, Assistant Entomologist; F. W. Terry, Assistant Entomologist. In August 1904, O. H. Swezey was engaged especially for breeding parasites and plantation inspection work, and later had charge of the distribution of parasites to plantations and the progress of their establishment and spread in the fields. Mr. Swezey was specially qualified for this work by his previous experience with leafhoppers and their parasites in Ohio. In the summer of 1905, soon after his return from the Australian leafhopper expedition, Mr. Koebele retired from active field work after a long career, leaving Honolulu not to return, and Frederick Muir was engaged for the continuance of foreign entomological explorations, for which he was well adapted.

Thus at its beginning and during its early years, the Division of Entomology at the Experiment Station, H. S. P. A., became equipped with a competent staff of entomologists (there were changes from time to time in later years) for coping with the leafhopper and other insect pests of the cane fields; the results of which have been in evidence in these later years when the plantations of Hawaii have been much less subject to insect depredations than other sugar cane countries. Indeed, some of the once-serious pests are so scarce as to be found only with difficulty on many of the cane lands of Hawaii.

#### THE PERKINS AND KOEBELE EXPEDITION TO AUSTRALIA

With the knowledge that the cane leafhopper had undoubtedly come as an immigrant from Australia, where it did no noticeable damage on account of probably being held in check by natural enemies, plans were made for an expedition to Australia for special study of leafhopper parasites after Mr. Koebele's return to Honolulu from his summer's work in Ohio in 1903. Mr. Koebele spent some time in California, where additional information was obtained concerning parasites of leafhoppers, before coming on to Honolulu early in 1904. The Australian trip started May 11, 1904, Dr. Perkins accompanying Mr. Koebele. The parasites which were discovered and successfully introduced are discussed in detail in subsequent pages. It will be appropriate to quote here from general remarks by Dr. Perkins (35, pp. iii-iv) on their itinerary, etc., which occupied the rest of the year:

Early in June we arrived at Brisbane and on the first cane that we saw, a few plants in the public gardens, we at once observed the presence of the cane leaf-hopper. A short stay of about ten days gave ample proof of the existence in Australia of a considerable variety of Hymenopterous parasites of leaf-hoppers, of Dipterous parasites of the genus *Pipunculus*, and of Stylopidae parasites of the genus *Elenchus*.

At Bundaberg, about twelve hours by rail north of Brisbane, we spent another ten days in June. Here is an extensive cane district with our leaf-hopper everywhere present, but never in numbers such as we are accustomed to in these islands. In fact we never saw the hoppers nearly as numerous as they are on our least affected plantations. From eggs collected here Mr. Koebele soon bred out specimens of the Mymarid parasites he had felt so confident of finding.

From our observations on the habits of the cane leaf-hopper in these islands, it seemed probable that in tropical Australia this species would be in its greatest numbers in the colder months, so after a brief stay in Bundaberg, we proceeded north to Cairns, which place we reached at the beginning of July. This plan seemed very expedient, for by retreating gradually towards the south, as the hot season advanced, we hoped to prolong the season during which natural enemies for the cane leaf-hopper could be obtained. It appeared likely that effective work could only be done at Cairns for a month or two, since without a reasonably large supply of hoppers, it was evident that the parasites could not be found in sufficient numbers for shipment. This indeed proved to be the case, and by the end of August, leaf-hoppers and their eggs had become so scarce in the cane-fields, that we came south again to Bundaberg. At Bundaberg we made a long stay on this occasion, regularly sending off consignments of parasites, until here too, owing partly to the season and partly to the harvesting of the crop, the locality became unprofitable. After a short stay in Brisbane, at the end of the year, I returned to Honolulu, while Mr. Koebele proceeded to Sydney, where his attention was largely given to collecting beneficial insects for pests other than leaf-hopper. On the return journey Mr. Koebele spent one month in Fiji, the enemies of the cane-hopper in those islands being mostly similar to those already found in Australia. A fine consignment of the Chalcid egg-parasite (*Ootetrastichus*) of leaf-hopper was most important, as it enabled us to establish that important species without any doubt.

## INTRODUCED PARASITES

**Paranagrus optabilis** Perkins (35, p. 199) (Mymaridae)

This is the most important of the several leafhopper egg parasites that were introduced. It was discovered by Koebele in June 1904, in the Queensland cane fields at Bundaberg. It was later found at other places in Queensland. In the first attempts at introduction, four consignments of this parasite were sent from Cairns, Queensland. The material sent consisted of small cuttings of midribs of cane leaves containing parasitized leafhopper eggs, packed in damp Sphagnum moss in small boxes, and placed in the cool-chamber on the steamer. Apparently none survived from these consignments. Subsequent consignments sent from Bundaberg were more successful, and a few parasites issued after arrival in Honolulu. It is not

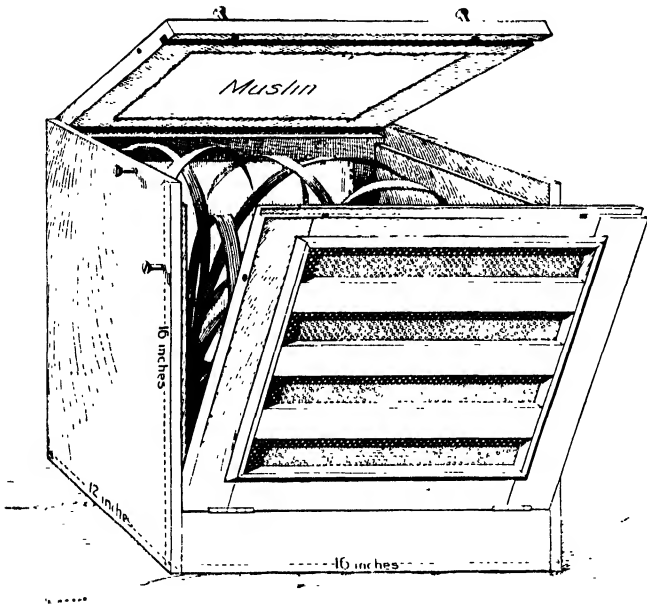


Fig. 1. Shipping cage for egg parasites.

certain that anything was accomplished however, until consignments were made in cages of living cane plants with leafhoppers breeding thereon and the parasites breeding in the leafhopper eggs. It is probable that this parasite became established from eight females which issued from one of these cages from Bundaberg. The cage (Fig. 1) arrived December 14, 1904. An occasional parasite issued at intervals. The eight females mentioned above were found in the cage by Dr. Perkins on January 26 and 27, 1905. Four of the parasites were liberated in the Experiment Station grounds, and four were transferred to a breeding cage consisting of a large glass battery jar (Fig. 2) containing a young, growing cane plant, in the leaves of which leafhopper eggs had previously been deposited.



At the end of three weeks the first brood of parasites began to appear, and, in all, 47 individuals, all females, were obtained. Half of these were liberated, the rest being used to stock a number of new breeding jars similar to the first. From these a very large number of individuals were reared and were treated in various ways. Some were "sleeved" out in the fields on growing cane containing many leafhopper eggs (Fig. 3). In the insectary large colonies were reared on large cane plants in tubs (Fig. 4), and still larger cages were used in the field where several leafhopper-infested stools of cane could be covered (Fig. 5). All of these methods were successful in breeding *Paranagrus* and other egg parasites as well.

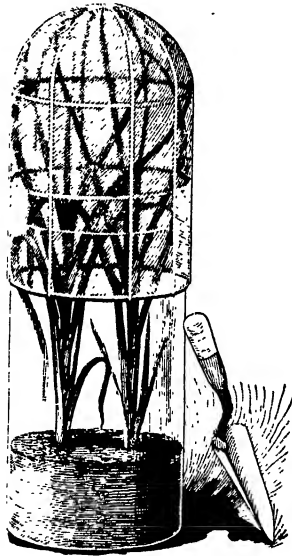


Fig. 2. Battery jar breeding cage for egg parasites.

While the parasites were still comparatively scarce and not easily obtainable in numbers for distribution, they were sent out in colonies in the large battery-jar cages mentioned above. Several of the plantations were supplied with these cages in April and May, 1905. The jar was placed by an entomologist in the desired cane field, properly protected from ants, a shade arranged for protection from sun and rain, then the cover of the cage removed so that the maturing parasites could go to the surrounding cane to parasitize the leafhopper eggs, which were numerous in the leaves. In this manner *Paranagrus* was first established on a number of the plantations. Subsequently, when the cane in the Experiment Station grounds became well stocked with the egg parasites, numerous colonies were sent out by using cuttings of midribs of cane leaves well filled with leafhopper eggs, many of which would be parasitized, placing the cuttings in a simple wooden cage to be suspended in the cane field where the parasites, which might issue daily for several days, could find fresh leafhopper eggs in the surrounding cane. By this method the parasite was

finally generally distributed throughout the cane districts during the latter half of 1905.

In some of the cane fields first colonized, the parasite was found already established the latter part of 1905. During 1906 it was found established and generally spread throughout the cane districts. In 1907, wherever leafhopper eggs could be found, a good percentage, often a high percentage of them were found to be parasitized by *Paranagrus*. The leafhopper had been greatly reduced due to the combined action of this and other introduced egg parasites, and other natural enemies already present in Hawaii.



Fig. 3. Sleeve cages in field, for breeding egg parasites.

*Paranagrus* was especially effective against the leafhopper on account of its short life cycle, which was less than half as long as the life cycle of the leafhopper—about 6 weeks in the latter and 3 weeks or less in the former. With each, the length of life cycle varies with the season of the year, being longer during the cooler part of the year and in the normally colder regions of the Islands. Successive broods follow throughout the year. In the insectary in July, adults issued in from 15 to 20 days after oviposition, an average of about 18 days, which would allow for about 20 broods per year. Some experimental breeding in the field at high elevations on Hawaii,



Fig. 4. Tub cages in insectary.



Fig. 5. Large cage over cane in field for breeding egg parasites.

2150-2650 feet, February to May 1918, indicated a life cycle lengthened from 42 to 73 days, or only 5 or 8 broods per year.

Another advantage is that *Paranagrus* is chiefly parthenogenetic, and breeding of this parasite in the insectary went on for 8 months, producing about a dozen broods, before any males were observed.



Fig. 6. Leafhopper on cane leaf, also *Paranagrus optabilis* in the act of ovipositing in inserted leafhopper eggs (highly enlarged).

The leafhopper places one to a dozen eggs in each egg puncture in the cane leaf, and while ovipositing (Fig. 6) the female *Paranagrus* may insert one of her eggs into each one of these eggs. Only one egg is inserted in each leafhopper egg, or at any rate, only one develops to maturity. The parasite larva develops right in the egg contents, there being just the proper amount for normal growth. Pupation takes place within the leafhopper egg shell, and when the parasite has matured it issues from the outer end of the egg shell, without gnawing a hole, as some of the other parasites do. Hence, it is not so easy to determine at a glance whether *Paranagrus* have issued from the leafhopper eggs in infested leaves. The leafhopper eggs when parasitized by *Paranagrus* have a pinkish color which changes to a lemon yellow when the developing parasite reaches the pupal stage. By splitting a leaf containing leafhopper eggs, it is easy to distinguish by this coloration how many, or what proportion are parasitized by *Paranagrus*; thus the percentage of parasitism could be calculated in the field. Another method of determining the percentage of parasitism was to take cuttings of midribs with leafhopper eggs, confine these in a glass jar for a time, and count daily the leafhoppers hatching and the adult parasites which issued.

It was found that *Paranagrus* would issue from leaf cuttings for a period of three weeks. Advantage was taken of this fact by several plantations to distribute *Paranagrus* on an extensive scale to new fields of cane. As soon as leafhoppers had migrated to these new fields and the young shoots were stocked with leafhopper eggs, these fields were supplied with numerous cages, regularly arranged, containing cuttings of midribs from fields where *Paranagrus* was known to be abundant. From these cages, which were arranged under a shelter, the parasites on issuing could parasitize the leafhopper eggs in the young cane and thus become established earlier, and sooner check the leafhopper, although it was always found that after the parasite was thoroughly established on the plantations they themselves were always able to migrate early to new fields, even when of great area, almost as soon as the leafhoppers had migrated there.

On several plantations buildings were erected as parasite hatcheries for obtaining *Paranagrus* in large quantities to be liberated in fields where the parasite was not sufficiently abundant to be effective, thus endeavoring to augment its usefulness. In these hatcheries, compartments were regularly supplied with cuttings of midribs from the cane fields where *Paranagrus* was most abundant. The compartments were darkened and adapted for accumulating the freshly issued *Paranagrus* adults in glass jars which could be taken to the desired fields for liberation of the parasites. They were handled by millions in this manner.

Notwithstanding these methods of distribution of the parasite, and the ability for dispersal of the parasite itself, and the fact that in most regions the leafhopper had become satisfactorily controlled, there were plantations on which, even 12 years after the introduction of this parasite, there were times and certain fields in which very severe outbreaks of leafhoppers occurred, resulting in diminished crops and great concern on the part of the plantation managers as to why the parasite failed in effectiveness in these instances. This concern resulted in search being made for more natural enemies of the leafhopper in 1919 and the ultimate introduction of the egg-sucking bug *Cyrtorhinus mundulus* in 1920. (See page 79.)

A species of *Paranagrus* not readily distinguished from *optabilis* was introduced from Formosa in February 1916 by Dr. Muir, who brought back cuttings of midribs containing leafhopper eggs. A notable distinction when the parasite was being reared for distribution was a greater proportion of males than had been noticed with *optabilis*. Breeding of this parasite was not carried on very long, but colonies were sent to a few plantations, among them the Hawaiian Agricultural Company. In 1918, when samples of infested cane leaves were received from that plantation for determination of the percentage of parasitism of the leafhopper eggs, *Paranagrus* issued abundantly from the material from their Wood Valley fields. These were 29 per cent males, which was a much higher proportion of males than we had known with *optabilis*, indicating that the Formosan *Paranagrus* had become established there. No further attention has been given it, however.

#### ***Paranagrus perforator* Perkins (35, p. 199)**

This egg parasite was described from Fiji, where it attacked leafhopper eggs laid in thick stems of grass, and was presumably introduced from there at the time

*Ootetrastichus beatus* Perkins was introduced, but detailed records were not made at the time. It became established, but not to any great extent and was recovered a few times. It had a preference for the leafhopper eggs that were placed in upper exposed internodes of the cane stalk. It was bred from eggs of *Aloha ipomoeae* Kirkaldy, a leafhopper on morning glory vines, in Makiki Valley near Honolulu, March 2, 1906. There appears to be no record of it since then. It is similar to *Paranagrus optabilis*, the chief distinction being the elongate ovipositor which extends beyond the apex of the abdomen for a length equal to all of the joints of the hind tarsi.

**Anagrus frequens** Perkins (35, p. 198)

This mymarid was the first to become established of the egg parasites introduced from Queensland in 1904, and became very abundant for a time after becoming widely distributed and dispersed. It finally almost entirely disappeared from the cane fields after *Paranagrus optabilis* became prevalent. It is smaller than *Paranagrus*, distinguished from it by antennal and other characters. The female parasitized more particularly leafhopper eggs that were placed in the blade of the leaf rather than in the midrib. On maturing, the adult *Anagrus* gnaws a hole through the epidermis of the leaf to make its exit. Often the holes were seen in clusters, indicating that a parasite had issued from each leafhopper egg in the cluster. When most abundant, 100 and more of these exit holes could be counted per leaf. This parasite parasitizes the eggs of the corn leafhopper to a considerable extent, and is thus still extant, but for some time now has been rarely seen in the cane fields.

**Ootetrastichus beatus** Perkins (35, p. 263)

This tetrastichine egg parasite was discovered destroying the eggs of the sugar cane leafhopper throughout Australia and Fiji. It was introduced from Fiji by Koebele early in 1905. This is a larger insect than the mymarids previously discussed. It oviposits in one egg of the leafhopper egg cluster. When the developing parasite larva has finished eating the contents of this one leafhopper egg it proceeds to eat the other eggs in the same cluster, thus the adult varies in size according to the number of leafhopper eggs its larva has had available for food. The full-fed larva has a tendency to burrow into the leaf tissue a little to one side for a pupal chamber. When the adult is formed, it gnaws a round hole through the upper surface of the leaf to make its escape. This exit hole is larger than that made by *Anagrus frequens*, and can also be distinguished from the latter as there is only one exit hole from a leafhopper egg puncture, whereas with *Anagrus* there is a cluster of smaller holes per egg puncture. Breeding is almost entirely parthenogenetic, a male being rarely seen. The length of its life cycle is about twice that of *Paranagrus*, being about 40 days, which is lengthened in cooler seasons or cooler regions. The adult is pale yellow or greenish yellow with a few dark markings on the thorax. Length variable, 1 mm. more or less (Fig. 7).

This parasite soon became established in the leafhopper-infested cane at the Experiment Station grounds in Honolulu, then it was distributed generally to the

plantations during 1906 in cuttings of midribs containing parasitized leafhopper eggs, these being sent out in wooden box cages to be suspended in a sheltered place in a cane field where the parasites that issued could find plenty of leafhopper eggs in which to breed. Many colonies were thus distributed. Apparently the first recovery on a plantation was at Olowalu, Maui, August 1906. In the summer of 1907, it was found to have become established on nearly all of the plantations and quite generally spread; on many of the plantations it was reported to be present wherever leafhopper eggs could be found. On a few of the plantations on the island of Hawaii it was not recovered until 1908. By this time it had become very abundant

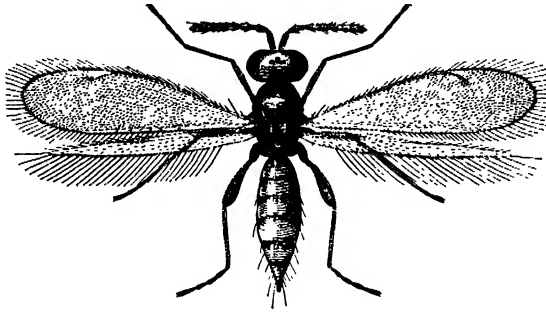


Fig. 7. *Ootetrastichus beatus*.  
(Courtesy of Territorial Board of Agriculture and Forestry.)

nearly everywhere, and often seemed to be doing more than *Paranagrus* in checking the leafhopper. It interfered somewhat with the effectiveness of *Paranagrus* for when its larva ate all leafhopper eggs of a cluster it no doubt often ate some eggs which were parasitized by *Paranagrus*. Its abundance could readily be determined by counting the exit holes in a leaf. It was common to find as many as 20 of these holes in a leaf, and counts were made of larger numbers. The highest count recorded was 79 exit holes in one leaf. As the average number of leafhopper eggs per cluster was over 4, the 79 parasites in developing would have destroyed over 316 leafhopper eggs. In recent years, the leafhopper itself being scarce, *Ootetrastichus beatus* is seldom seen.

#### ***Ootetrastichus formosanus* Timberlake (55, p. 558)**

This parasite was found by Dr. Muir parasitizing eggs of a leafhopper in cane leaves in Formosa. There are several species of leafhoppers on sugar cane in Formosa, and it is not recorded just which species was the host of this parasite. Dr. Muir brought a quantity of midribs upon his return to Honolulu in February 1916. From these midribs about 20 females and a few males of *Ootetrastichus* issued. They were placed in breeding cages where they reproduced on the eggs of the sugar cane leafhopper and, after a few generations, became numerous enough for liberation in cane fields. Multiplication of the parasite was carried on in breeding cages in the field at the Experiment Station during 1916 and 1917, and it was distributed in large numbers to the sugar plantations on the different Islands. During the latter

half of 1917 (June to December) it was found established in fields of six plantations on Oahu, Maui and Hawaii. In 1918 it was recovered on 23 additional plantations on Oahu, Kauai and Hawaii. On some of these plantations it was found to be very abundant and colonies were obtained from these fields for further distribution to other plantations. Finally, in 1919, it was recovered from 7 additional plantations, which indicated nearly complete establishment throughout the plantation districts. It soon became so abundant as to appear more effective than *Ootetrastichus beatus*.

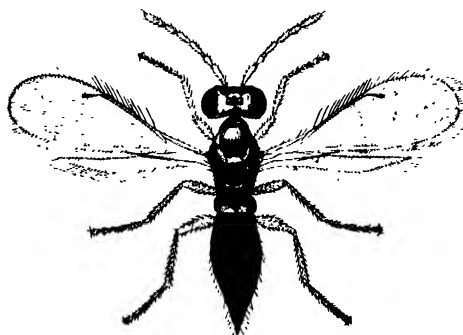


Fig. 8. *Ootetrastichus formosanus*, female.

At the Waipio substation in May 1918, as high as 37 per cent of the leafhopper eggs were found to be parasitized by this parasite, which was a higher rate than by the other egg parasites at the same place and time. Conditions were found similar in several other localities. In later years when the leafhopper became universally scarce, *formosanus* was found oftener than *beatus*.

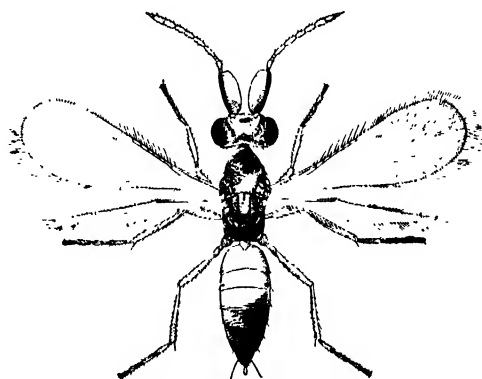


Fig. 9. *Ootetrastichus formosanus*, male.

*Ootetrastichus formosanus* is of darker color than *beatus*. The female (Fig. 8) is about 1.25 mm., varying with the number of leafhopper eggs in the egg cluster which the larva had the opportunity of eating. The thorax and hind part of the abdomen are metallic greenish, the basal part of the abdomen yellowish. The males (Fig. 9) are smaller and paler than the females and are usually about as many in number, or more. They are readily distinguished by the greatly enlarged basal joint of the antenna. In breeding experiments the life cycle (egg to maturity) was found



to be—egg and larva about three weeks, pupa about ten days—31 days with a minimum of 24 days, varying between that and two months. The adults were found to live as long as one to two months. In two experiments as to fecundity 39 and 95 respectively were produced from single females. In both cases the male progeny outnumbered the female.

**Haplogonatopus vitiensis** Perkins (35, p. 488)

This dryinid was first bred from a delphacid on a grass, *Zoysia pungens*, in Fiji, by Mr. Koebele in March 1905. A single female issued from a cage received March 7, 1906, from Fiji, sent by Dr. Muir in an attempt to introduce a stylopoid parasite on the leafhopper. This female was placed in a cage with young leafhoppers which it instantly seized and apparently parasitized; thus, breeding of the parasite was started. Multiplication was slow, and only a few small colonies were distributed from July 1906 to August 1907, to a few of the plantations on Oahu and Hawaii. From these colonies the parasite became established so that it was recovered in a few places in 1908 and soon became quite common in some regions. Later on, it became widely spread, and was recovered also from plantations on Kauai and Maui. For a time it was a factor in reducing the leafhopper pest, but by about 1918 it became so scarce as to be seldom seen. This was partially due to the diminished numbers of the leafhoppers, but for the most part was due to the work of hyperparasites on it. As an example of the prevalence of the hyperparasites, at Hilo Sugar Company 11 cocoons of *Haplogonatopus vitiensis* were collected on cane leaves February 27, 1919. Hyperparasites issued from all of them. There were: 4 *Echthrogonatopus hawaiiensis*, 24 *Helegonatopus pseudophanes* and 2 *Saronotum americanum*. A few years earlier the conditions were the same at the Experiment Station grounds in Honolulu. Of 9 cocoons collected March 7, 1913, hyperparasites issued from all of them: 1 *Saronotum australiac* and 28 *Ielegonatopus pseudophanes*. For several years past *vitiensis* has been seldom seen. The latest collected specimens in our cabinets were from the Kailua substation in 1929 and 1931. In 1931 when there was an outbreak of taro leafhopper in the Waianae district, *vitiensis* was found more abundant on this leafhopper than it had been for several years. (It was again recovered on cane leafhoppers at Kailua in January 1936.)

Only the female of this parasite is known, hence, breeding is parthenogenetic. The adult is always wingless; brown to brownish-black in color and about 3 mm. long. Usually it is the young leafhoppers which are attacked, but occasionally the adult leafhopper is parasitized. This is fortunate as it helped in dispersal, for these parasitized adult leafhoppers could fly to other regions before their death. As with other dryinids the larva feeds externally at the base of the abdomen, appearing like a wart on the body. With this species it is pale, about the color of the body of the leafhopper instead of being black as in the Fairchild parasite and the Chinese dryinid. When the parasite larva becomes fully grown it withdraws and leaves the empty leafhopper skin in order to spin a white oval cocoon on a cane leaf or stalk. The adult issues from the cocoon in about 2 weeks.

**Pseudogonatopus hospes** Perkins, The Chinese Dryinid (37, pp. 12-13)

This large black dryinid parasite of the sugar cane leafhopper was discovered by Dr. Muir at Wei Chou, about 120 miles up the East River from Canton, China, in

September 1906, where it parasitized a species of leafhopper (*Perkinsiella sinensis* Kirkaldy) on sugar cane. These leafhoppers were very scarce there, and only a very few were found parasitized by this dryinid. These were taken to Macao, China, where they were bred on leafhoppers in cages on sugar cane growing in tubs, until they had increased in sufficient numbers to forward to Honolulu. Shipments of cocoons of the parasite were received December 30, 1906, January 17 and February 19, 1907.

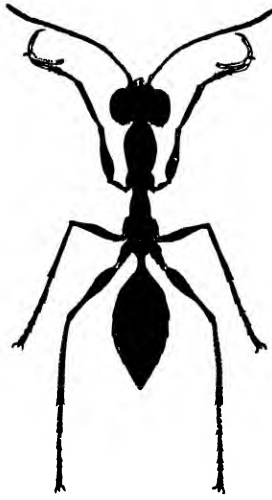


Fig. 10. *Pseudogonatopus hospes*, female.

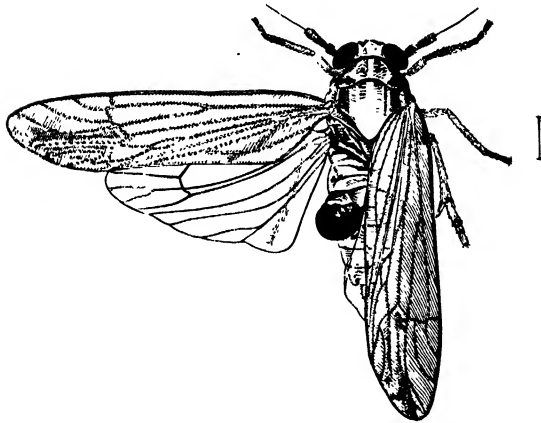


Fig. 11. *Pseudogonatopus hospes*, larva on leafhopper.

The adult parasites issuing from these cocoons were retained for breeding at the Experiment Station. They increased so slowly that it was not until June 1907 that the first colony was sent out. Other colonies were sent out for liberation during July and August. These were mostly to the plantations of Oahu, but a few colonies were sent to plantations on the other Islands also.

At best, only small colonies were liberated in any place, and it was thought later that this parasite had failed to become established for, during several years, none was observed. The first recovery was that of a single female found at the Waipio substation, Oahu, March 24, 1916. In April it was collected on the plantation of Oahu Sugar Company, Ltd., and in May three adult leafhoppers parasitized by this dryinid were collected on the same plantation. In June of the same year it was noticed quite commonly in the cane at the Experiment Station grounds in Honolulu.

During 1917 recovery of the parasite was made at Ewa Plantation and Honolulu Plantation on Oahu; Makee Sugar Company, the Koloa Sugar Company, Kekaha Sugar Company, Ltd., and Waimea Sugar Mill Company, Ltd., on Kauai; Pioneer Mill Company, Ltd., Wailuku Sugar Company, and Hawaiian Commercial and Sugar Company, Ltd., on Maui; Hawi Mill and Plantation Company on Hawaii.

During 1918 it was recovered at Kahuku Plantation Company, Oahu; Kaeleku Sugar Company, Ltd., Maui; Olaa Sugar Company, Ltd., and Hawaiian Agricultural Company, Hawaii.

During 1919 it was recovered at Waialua Agricultural Company, Ltd., Oahu, and Onomea Sugar Company, Hawaii. Thus, it had become generally spread throughout the sugar plantation districts. In 1929 it was found to have reached Molokai by itself. It never became very abundant in the cane fields, ordinarily not more than

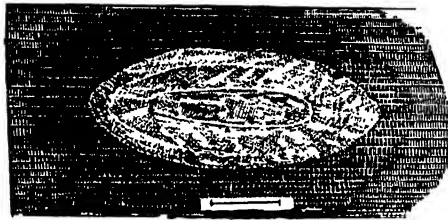


Fig. 12. *Pseudogonatopus hospes*, cocoon on leaf.

1 per cent of the adult leafhoppers would be found parasitized. The highest records taken were: 9.21 per cent on one occasion at Ewa Plantation Company in 1918, and 13.31 per cent at the same plantation in 1919. Hyperparasites\* were found at work, so that when the dryinid cocoons were collected in the field, hyperparasites issued from a large proportion of them. From counts taken at various times of cocoons collected in the field, 25 to 100 per cent were found to be affected by one or more of the hyperparasites. This of course prevented this parasite from reaching any important efficiency. However, it has persisted even to recent years and with the scarcity of leafhoppers. Wherever a few of the latter are to be found, there is also likely to be found an occasional parasitized leafhopper, or an adult or cocoon of the parasite.

This dryinid attacks the adult leafhopper (Fig. 10). Only rarely is an im-

\* *Helegonatopus pseudophanes* Perkins, *Saronotum americanum* Perkins, *Ceraphron abnormis* Perkins and *Paraphelinus xiphidii* Perkins.

mature leafhopper found parasitized by it. In this respect it differs from the Fairchild parasite (*Echthrodelpfax fairchildii*) and the Fiji dryinid (*Haplogonatopus vitiensis*), both of which attack the immature stages of the leafhopper and only rarely an adult.

In parasitizing a leafhopper, the parasite catches and holds the leafhopper with her forelegs, which are especially adapted for this purpose (see Fig. 10), while she inserts the egg in the dorsal part of the abdomen. Having accomplished this, the leafhopper is released and it goes on about its usual life while the parasite develops on its back, resembling a black wart (Fig. 11). When the parasite larva becomes full-grown the leafhopper dies, and the parasite larva spins a white, oval, flat cocoon on a leaf (Fig. 12) or on the sugar cane stalk itself. The larva obtains its growth in one to two weeks. About three or four weeks are spent in the cocoon before the emergence of the adult. Thus, the life cycle is four to six weeks which is approximately about the same as that of the leafhopper.

In the laboratory a female dryinid lived 37 days and parasitized 153 leafhoppers, indicating the value of the parasite if its increase were not checked by the hyperparasites. The sexes are about equal in numbers, the male being winged while the female is always wingless (Fig. 10), although in one lot of 45 reared in the insectary there were 16 females and 29 males. In the field the female was more often observed. She would be greatly handicapped in accomplishing dispersal if it were not for the fact that it is the adult leafhoppers which she parasitizes. They have been found established early in newly planted fields at some distance, where they could not have migrated except by means of parasitized leafhoppers migrating by flight to the place before they were killed by the parasite larvae which they were carrying.

### ***Cyrtorhinus mundulus* (Breddin) (1)**

While in Queensland in 1920 in search of additional natural enemies for the sugar cane leafhopper, Dr. Muir discovered that the little mirid bug, *Cyrtorhinus mundulus*, had the habit of piercing and sucking leafhopper eggs, and was the most efficient control agent of that pest. Although belonging to a family of bugs which are chiefly plant feeders, it seemed never to suck plant tissues. A small colony of the bugs was brought to Honolulu, and later in the year larger consignments were obtained and sent from Fiji by C. E. Pemberton. The bug had previously been known by Dr. Muir in Fiji cane fields without his having learned its habits. Three consignments were received from Fiji in September, October, and November 1920, and consisted of adults and young in cages with growing cane and leafhoppers. Several hundreds of the bugs were received in this manner. Some were released in plantation fields infested with leafhoppers, others were used for breeding in cages; breeding was kept up for a year. From the breeding cages many hundreds of bugs were obtained for distribution to the regions where the leafhoppers were most abundant. The bug readily became established in these places and spread from them throughout the entire sugar cane areas and even reached Maui and Molokai

without assistance. The first recovery was at Olaa only a month after liberation (their eggs were found in leafhopper-infested cane leaves sent in for examination). During the following year (1921) a few scattering recoveries were made and it seemed doubtful if the bug were becoming established sufficiently to be of any im-

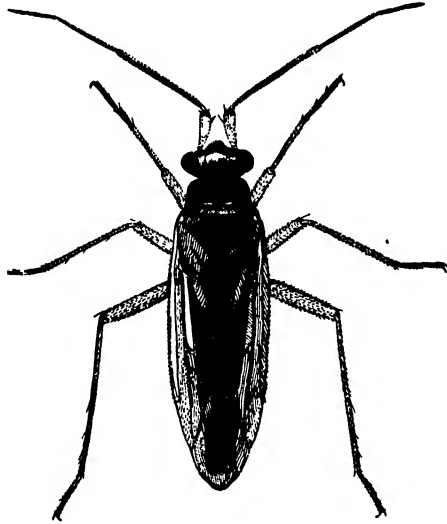


Fig. 13. *Cyrtorhinus mundulus*, adult.

portance. In March 1922, the bugs were found very abundant at Ewa Plantation, at Waialua and at Olaa. During the year it was found sparsely in many regions, and during 1923 was found to be generally distributed throughout all the cane regions. The leafhopper was now almost entirely reduced, this bug proving to be more efficient in destroying the leafhopper eggs than were the egg parasites. In fact, without doubt, *Cyrtorhinus* caused a reduction in the efficiency of the egg parasites for it



Fig. 14. A. An egg puncture of the leafhopper in cane leaf. B. The tips of two *Cyrtorhinus* eggs in an old leafhopper puncture.



Fig. 15. Midrib of cane leaf sectioned showing two *Cyrtorhinus* eggs in an old leafhopper puncture.

sucked leafhopper eggs regardless of whether they were already parasitized or not. In a few more years, with the scarcity of the leafhoppers, it became difficult to find

the egg parasites in the fields or parasitized leafhopper eggs. At this time (from 1923 on) the control of the leafhopper was considered to be complete, having finally reached this condition through the introduction and establishment of the *Cyrtorhinus*, which had increased to great abundance wherever there were leafhopper eggs. As outbreaks of leafhoppers were reduced by the *Cyrtorhinus*, the latter disappeared also, to appear again and increase to abundance wherever any new outbreaks of leafhopper occurred. It was considered by the entomologists (63) that if this bug had been the first to be introduced, it would by itself have been sufficient for the control of the leafhopper. Dr. Williams (63, pp. 103-104) states:

The adult *Cyrtorhinus* [Fig. 13] is about 3 millimeters long; the general color is black, with the body in part (beneath the wings) reddish in the males and in all young adults, the legs and the base of the antennae are pale and the light smoky wings have a broad whitish front border. It seeks the eggs of the leafhopper and sucks them through a minute puncture which it makes with its slender beak. Wary and exceedingly active, it is usually approachable only with caution, otherwise it will dodge behind a leaf or stem or make a hasty flight to the next plant. The eggs [Figs. 14, 15] are inserted into small crevices in the cane leaf, a leafhopper egg-slit being frequently chosen; they are of shorter and stouter form than those of the leafhopper and occur singly or in very small groups. Rather close scrutiny is required for their discovery, when they may be recognized, where they are exposed, flush with the surface of the leaf, as rather evenly oval white discs or caps, the center of which is sunken and dark giving them a ring-like appearance in contrast to the irregularly protruding, waxy covering that protects the tips of the leafhopper eggs. [The eggs are white, becoming bright red before hatching.] The young *Cyrtorhinus* are rather short, and bright red and suggest somewhat red spiders or mites of the genus *Trombidium*. . . .

Their favorite habitat is within the spindle of the cane plant and when very numerous they were also found among the bristles of the leafsheath. Under favorable conditions *Cyrtorhinus* may produce ten generations per year.

#### NATURAL ENEMIES WHICH WERE ALREADY PRESENT IN HAWAII

In the years when the leafhopper infestation was at its maximum, it furnished an abundant food supply for numerous insects already present in Hawaii, the most of which were predatory in nature, although a few were parasites. Some of these insects were native species inhabiting the forests in the vicinity of cane fields, in which case they migrated into the cane fields where their food was plentiful, and they themselves increased to greater numbers than ever existed in their natural habitat. Several species of spiders, too, increased greatly in cane fields where unlimited supplies of food were found when leafhopper infestations prevailed.

Taken altogether, these had an appreciable effect on the reduction of the leafhoppers, and should be given ample credit for their part in the final results. Some account of these insects is given in various publications and by different entomologists: Perkins, Kirkaldy, Terry, Swezey, Muir, and Williams.

#### **Echthrodelpax fairchildii** Perkins (32, p. 37)

This dryinid (Plate 1) was discovered in 1903 parasitizing cane leafhoppers on Kauai, by Geo. H. Fairchild for whom it is named. Dr. Perkins considered it to be

a native species which had normally attacked native leafhoppers, but had transferred its attention to the cane leafhopper when the latter became abundant and furnished a great deal of host material for it. Many thousands of the parasites were reared in Honolulu during 1903 and 1904 and distributed to the plantations of the other Islands. It readily became established and common and was considered a help towards the reduction of the cane leafhopper before any of the egg parasites were introduced from Australia. It practically disappeared a few years later, and has not been seen for over 20 years. There are no specimens in our cabinets of a date more recent than 1905.

Both sexes are winged; the male is black and smaller than the female; the female black with yellow markings, and is 2.5 mm. long. Only the young leafhoppers are parasitized by this dryinid. The method of attack, etc., is given in detail by Dr. Perkins (35, pp. 7-10) as follows:

When in 1903 for the purpose of distribution in the canefields many hundreds of *Echthrodelpfax* were kept in a cage with glass sides and large enough to contain a fair-sized growing plant of sugar cane, on which large flocks of the larvae of the cane leaf-hopper were feeding, the habits of the parasite could be studied to great advantage. By having a cage thus well stocked with the parasites, one can insure the fact that at almost any time individuals may be seen in the act of catching their prey. In such a cage, on one occasion, I counted over thirty parasites on a single cane stem each one simultaneously engaged in stinging the young hopper it had seized. When the hoppers were excreting an abundance of honey-dew, the parasites fed freely on this, but if not, some sweet liquid was supplied in place of it. Without liquid food, in a hot locality the parasites die very quickly, and the cage was freely sprinkled with water each day to advantage. Pairing of the sexes is of short duration and after copulation the male frequently never moves again, and in general dies very quickly. To watch the female parasite stalking, catching and stinging its prey, is a most interesting sight. The prey is sought on foot, for while most of the Dryinidae are most active and rapid runners, they are but poor performers on the wing. In most of the winged forms, these organs are unduly short and in *Echthrodelpfax* serve hardly more than to transport it from one cane plant to another as occasion demands. As soon as the parasite becomes aware of the presence of its prey, it usually comes to a standstill, while still at a short distance; it assumes a rigid attitude comparable with that of a dog pointing game; the antennae are laid back behind the head; frequently it sidles round the hopper to gain a more advantageous position for the attack. The hoppers often show manifest uneasiness on the approach of the parasite and they hasten to remove themselves to a distance, as the latter comes to a point. In this case they are again followed up, and the performance may be repeated several times. In some cases through too great deliberation in attack the prey is entirely lost, as it moves away into concealment and the parasite fails to trace it up. Deliberate as it often is in making the attack, yet, when made, the stroke is marvelously rapid. So quickly indeed are the front legs thrown out and withdrawn that the hopper, which just now was at a distance, in an instant appears contiguous to the parasite, as if attracted by some unseen force. One pair of pincers usually grips the neck of the prey the other frequently clasps the pair of hind legs in the neighborhood of their long jumping spurs, or the abdomen towards the apex. If the hopper is unusually large and strong compared with its enemy, it not rarely manages to make its leap, and both fall to the ground together. Never however was the latter seen to relinquish its hold on the former. Its prey firmly secured and frequently held more or less crosswise to itself, the parasite now curls round the abdomen and thrusts its sting into the side of the hopper, beneath one of the wing lobes in the case of *Echthrodelpfax*, and in various other positions in the case of other parasites, and the egg is deposited. The laying of the egg is again a very deliberate undertaking and the sting may remain inserted for a couple of minutes or more. Finally the sting is withdrawn, the front leg that grasps the hopper's neck is extended, the chelae or pincers fly open and the hopper is sometimes roughly jerked to a distance, sometimes more gently deposited on the plant. While grasping the hopper and in-

serting the sting, the parasite has been seen in some cases to freely use its mandibles on the neck in process of malaxation. After the operation, the victim usually appears weak and dazed, sometimes even lying inert on the ground, but sooner or later and sometimes very quickly, it recovers and starts feeding as if nothing had happened. Occasionally after capture, the prey is released without being stung, and it is probable that hoppers so released have already been stung by an earlier captor. Under unnatural conditions, such as in the confinement of a small jar or glass tube, and probably under pressure of hunger, the hoppers are frequently killed outright, and to some extent devoured. The position of insertion of the sting is apparently not always the same, this being sometimes inserted beneath the wing-lobe, and sometimes in the ventral side of the body, but the larval sac in either case appears beneath the wing-lobe. After the egg has been deposited, it is not for some time that the characteristic larval sac becomes evident externally. In the case of *Echthrodelpfax*, I could distinguish the larval sac, having the appearance of a minute transparent vesicle, at the end of four days with the naked eye. In the case of a California species of *Haplogonatopus*, the period was not less than a week. On one occasion three cane leaf-hoppers were placed in a large glass jar with the *Haplogonatopus*, and two of them were seen to be quickly seized and stung. The next day the parasite was removed to another cage. At the end of six days, when the hoppers were examined, no sign of the larval parasite was noted, even with the aid of a weak lens, and it was supposed that they were unaffected. However on the ninth day, when they were again examined, the parasitic larvae were of considerable size, and obvious to the naked eye. The third hopper of the above produced no parasite and probably was not stung. As soon as the larval sac becomes visible, it is usually but a short time, a few days or a week before the larva becomes mature. The length of time no doubt varies somewhat according to the species, and according to climatic conditions.

The larva of the *Echthrodelpfax fairchildii* while still attached to the hopper, appears as a small, nearly circular, impressed, black object, adherent to the young leaf-hopper. The latter seems hardly to be inconvenienced by the parasite, remaining as active and plump as the non-parasitized individuals. It is always the immature hopper that is attacked and a single hopper may sustain one or two parasites. They are generally fixed beneath the lobes, which develop into the tegmina or upper wings, one on each side of the body, if two be present; they are, however, sometimes found beneath the true wings.

After a time, however, (always shortly before the full growth of the parasitic larva) the hopper becomes sluggish and then entirely stationary. This may happen either shortly before or not till some time after the black shell-like covering or larval sac of the parasite splits by a longitudinal (mediodorsal) fissure and exposes the back of the white maggot within. This torpidity of the leaf-hopper and the splitting of the covering of the parasite is the outward sign of a change of habits in the latter (being coincident with a moult and change of form of the parasite). From this time until the hopper dies and the maggot finally quits hold of its prey the sight as examined under a lens forms one of the most repulsive sights that natural history can afford.

Soon after the splitting of the black covering and the exposure of the white maggot, a conspicuous change takes place in the color of the latter, it becoming pink or reddish. The maggot, which has hitherto fed delicately without doing any vital injury to its host, now proceeds to ingest the contents of the hopper in an indiscriminate manner, and the change in color is clearly due to this. If removed at this time from the hopper it is seen to have very mobile and hard (chitinated) mouth parts, while the thin and collapsed black covering still adheres some distance behind the head. Growth is extremely rapid and the simultaneous shrinking of the hopper, as its contents are absorbed by the parasite, enhances this effect. Thus when the splitting of the black covering takes place the hopper may be three or four times the size of the parasite, when the latter is full fed the proportions may be exactly reversed. The removal of the contents of the hopper can be easily seen through parts of the cuticle. Generally early in the proceedings the soft contents of one or both eyes and of the head are seen to be in rapid motion, like a boiling fluid, suddenly all the pigment is removed from one eye (usually the one on the opposite side to the parasite) and it becomes an opaque white spot, then the other is often similarly destroyed, or sometimes both more or less simultaneously.



Finally the maggot, when it has finished feeding, withdraws its head, and may then sometimes be seen busily engaged in applying sticky matter from its mouth to its body. Its surface is strongly adhesive and when it quits its prey, it is able (though of course quite legless) to crawl freely over any surface, however smooth. Soon it spins a neat white cocoon, from which it emerges as an active winged insect in about 18 days.

There is another native species of the family Dryinidae, *Pseudogonatopus perkinsi* (Ashm.), which parasitizes several species of delphacid leafhoppers in the native forests of the Hawaiian Islands, but it was never found to attack the cane leafhopper.

### **Coelophora inaequalis (Fab.)**

This lady beetle (Fig. 16) was introduced from Australia by Mr. Koebele, in 1894, as an aphid-feeder. It soon increased until it was generally spread and at the time of maximum leafhopper infestations was found to be nearly always present in leafhopper-infested cane fields and sometimes extremely numerous. Its larvae took to feeding on the young leafhoppers, especially at the moment of hatching, and became very efficient.

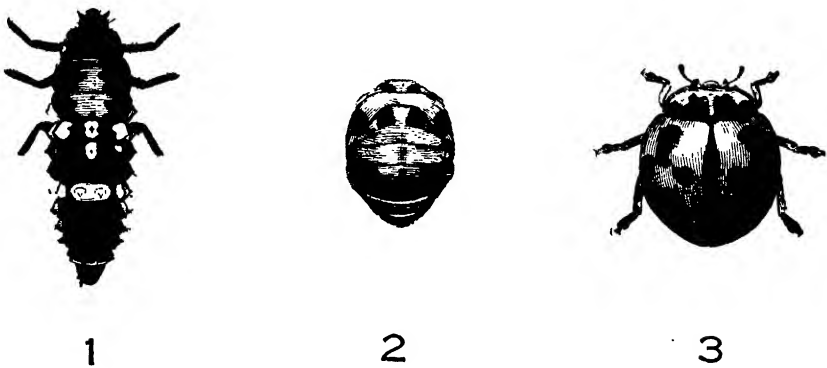


Fig. 16. Australian ladybeetle, *Coelophora inaequalis*.

1. Larva.

2. Pupa.

3. Adult.

The life cycle of this lady beetle is short, being 2 to 3 weeks under favorable circumstances and with plentiful food supply. The egg stage is 3-4 days, larval stage 8-9 days, pupal stage 3-4 days, totalling 14 to 17 days. Adults are notably long-lived as with many lady beetles. The voracious nature of the larvae together with the short life cycle, by which a rapid increase in numbers takes place, make this a very valuable leafhopper enemy. The adults quickly found leafhopper outbreaks at their beginning, and could soon increase to efficient numbers. The fact that they fed primarily on aphids was a help toward their maintaining their existence in cane fields, as there were likely to be enough cane aphid infestations, generally distributed, so that the population of lady beetles could be maintained on one or the other and always be ready to migrate to new outbreaks.

At the present time this lady beetle is scarce in cane fields, as there are less outbreaks of cane aphid and numerous natural enemies check these soon enough to

prevent severe infestations, and the leafhoppers are so scarce that probably they are no longer of any importance as food for the larvae of this lady beetle. In gardens, this lady beetle is always to be found when aphid is present on any plants. They are especially noticeable on hibiscus hedges when infested with the cotton aphid.

A braconid parasite, *Dinocampus terminatus* (Nees), has occasionally been found attacking the adult of this lady beetle, but it is never numerous enough any more to be of importance in the effectiveness of the lady beetle.

### **Conocephalus saltator** (Sauss.)

This is commonly known as the longhorned grasshopper. It was described by Mr. Swezey as *Xiphidium varipenne* (41, p. 216) on account of the variation in length of tegmina, but later was found to be of tropical American origin and is now known as *Conocephalus saltator*. It was known as an immigrant insect in Hawaii as early as 1895. In 1899, Dr. Perkins stated of it in the *Fauna Hawaiiensis*, "Only in and around Honolulu." It must have increased and spread rapidly soon thereafter for Mr. Swezey says of it in 1905, "At present very generally distributed, in fact, it might be said that they are everywhere, lowlands, valleys and mountains, gardens, pastures and cane fields." They became especially abundant in leafhopper-infested cane fields. As there was no evidence of their being injurious to the cane, particular study of their habits revealed that they were feeding extensively on the leafhoppers. They had the habit of lurking about the axils of the upper leaves, or the crown of the cane stalk just where the leafhoppers congregated in large numbers in the young cane, and it was easy for the grasshoppers to capture an abundance of their prey, especially the young leafhoppers. In cage experiments grasshoppers would scarcely eat any of the cane leaves, but would readily devour leafhoppers that were introduced. Freshly hatched grasshoppers starved to death in 5 to 6 days in the presence of tender grass and cane leaves, while those provided with leafhoppers were reared to maturity. Older grasshoppers do nibble the tips of cane leaves to a slight extent. They also feed on flowers of various kinds, as canna, lantana, ginger, morning-glory and many weeds. Sometimes this grasshopper has done considerable injury to rice by feeding on the heads when the growing rice grains were still soft.

The female (Plate 3) has a sword-like ovipositor by which she inserts her eggs behind the leafsheath on cane and in similar places on grasses, canna, or other plants. The eggs are 5 mm. long and are deposited in clusters of 2-15, side by side, with the anterior end outermost. They are white, turning green before hatching, which occurs in about 5 weeks. The young grasshoppers molt at intervals of 6-23 days during their growth, becoming mature on the sixth molt. This takes about 10 weeks or a total of about 15 weeks from egg to adult. It is not known how long an adult lives, but it is certain that one individual during its period of growth and then its mature period would consume many hundreds of leafhoppers, no doubt thousands of them. As there were swarms of all stages of the grasshoppers in cane fields when badly infested with leafhoppers, it is evident that these grasshoppers would be of great service in reducing the leafhoppers. Since the cane leafhopper has become scarce, this grasshopper is no longer abundant in cane fields, but is always to be found in grassy regions, where it undoubtedly finds insects to prey upon, but no

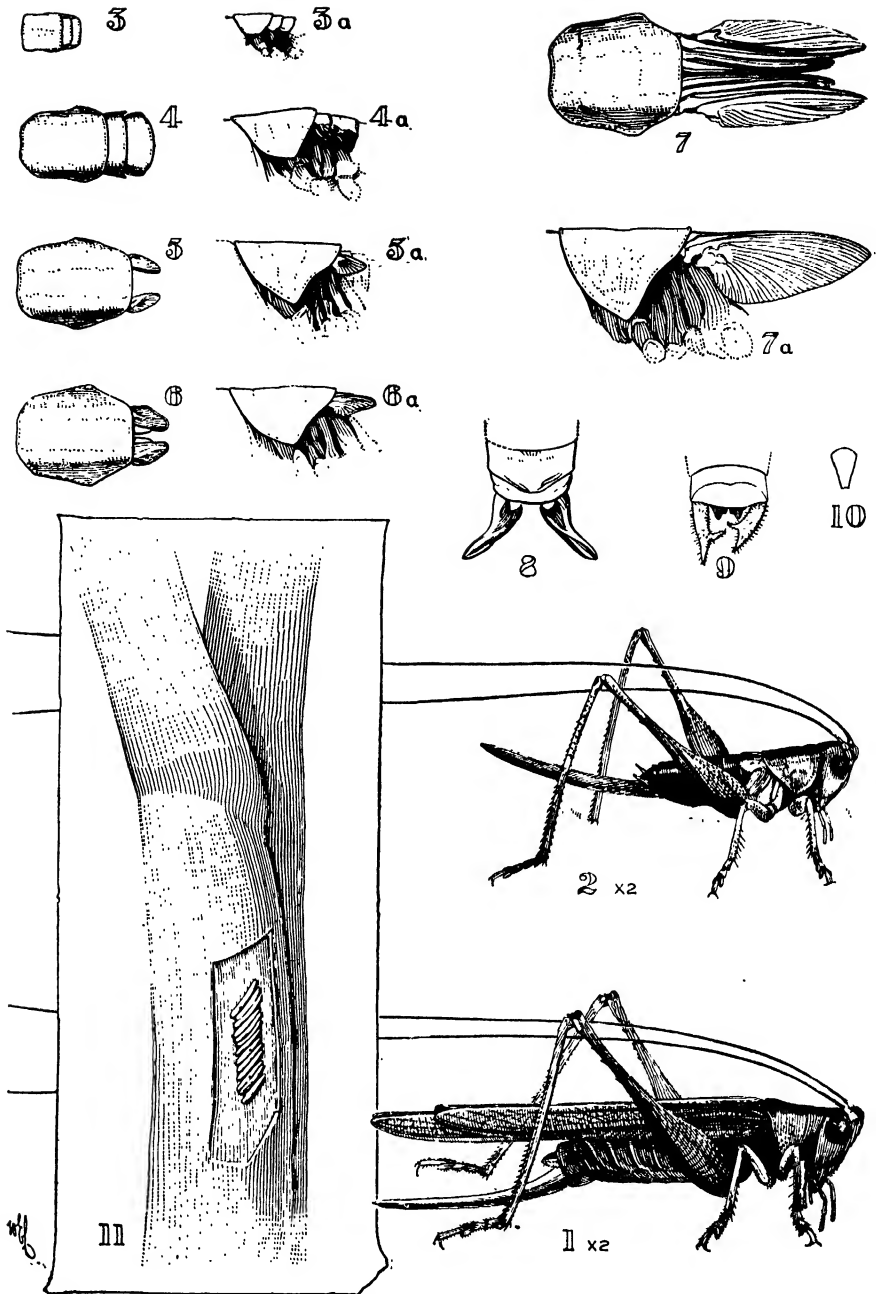


Plate 3. Long-horned grasshopper (*Conoccephalus saltator*).

1. Adult female.
2. Immature female.
- 3-7 and 3a-7a. Dorsal and lateral views of thorax to show development of wings.
8. Cerci of male.
9. Cerci of *C. fuscum*, a related species not found in Hawaii.
10. Fastigium (an area on top of head).
11. Eggs, natural size, showing location behind leafsheath of sugar cane. The tiny holes in several of them show where parasites have issued.

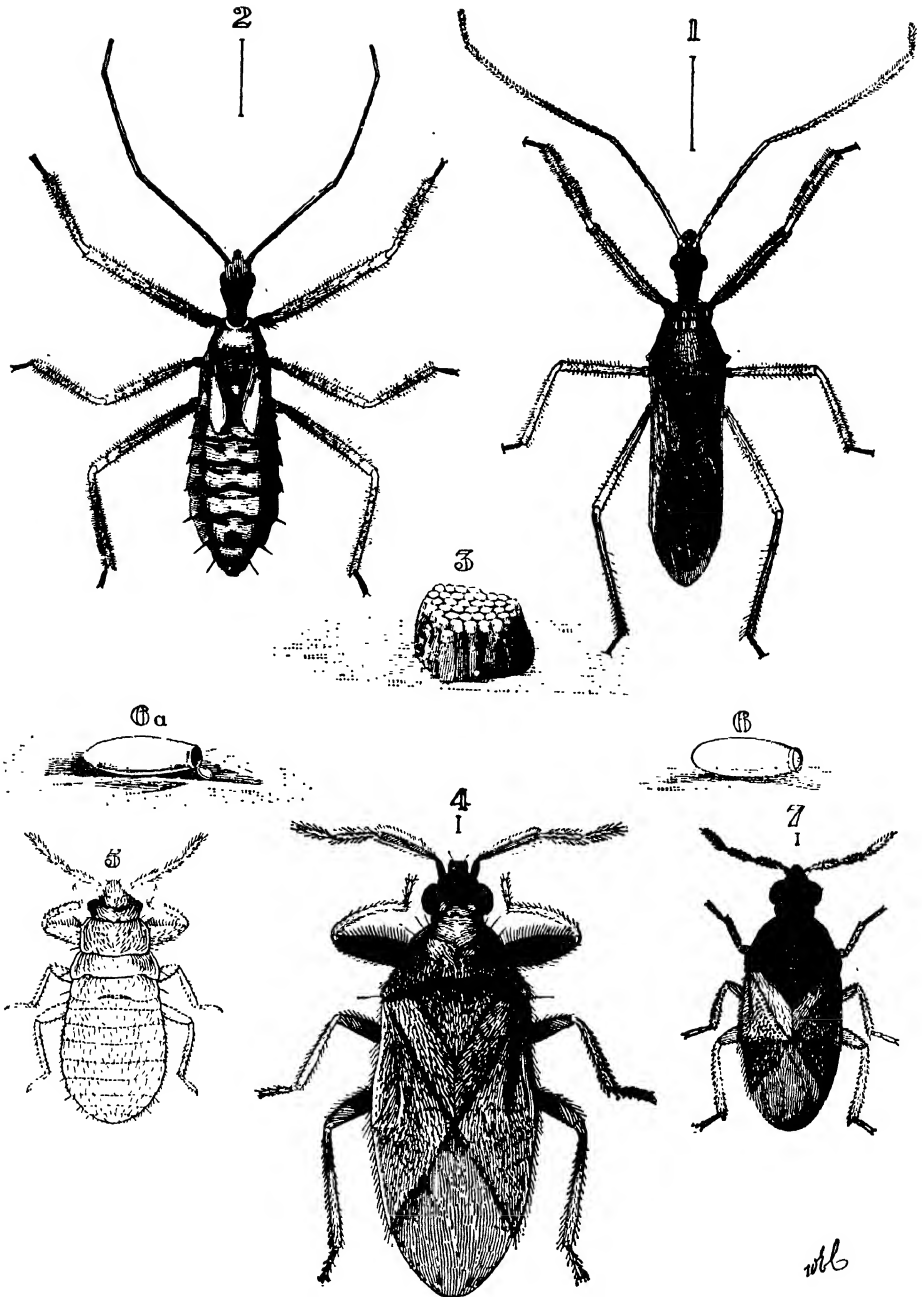


Plate 4. Predacious bugs.

1. *Zelus renardii*, adult, x 4.
2. *Zelus renardii*, nymph, x 4.
3. Egg cluster, x 5.
4. *Physopleurella mundulus*, x 20.
5. *Physopleurella mundulus*, nymph, x 20.
- 6 and 6a. *Physopleurella mundulus*, eggs, x 15.
7. *Triphleps persequens*, x 20.

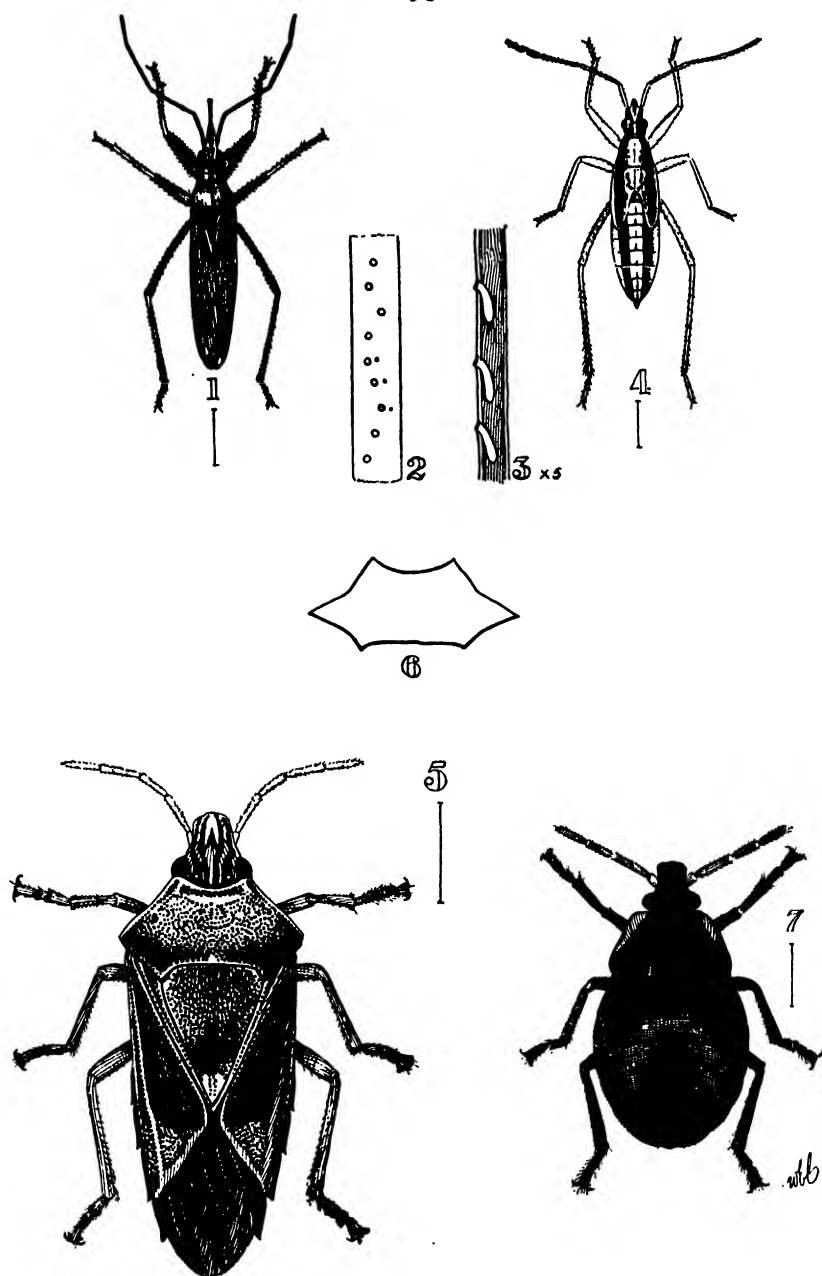


Plate 5. Predacious bugs.

1. *Reduviolus blackburni*.
  2. *Reduviolus blackburni*, surface of leaf showing external appearance of eggs which are inserted; the tiny holes at the side of several are where parasites have emerged.
  3. *R. blackburni*, section of leaf showing inserted eggs.
  4. *R. blackburni*, nymph.
  5. *Oechalia grisea*.
  6. *Oechalia grisea*, prothorax of another specimen showing variation in the spines at posterior angles.
  7. *O. grisea*, nymph.
- All figures  $\times 5$ .

***Triphleps persequens*** White (59, p. 111)

***Physopleurella mundulus*** (White) (59, p. 111)

These two small anthocorid bugs (Plate 4, Figs. 4-7) are more especially predacious on aphids, psocids and other small insects. They are considered as immigrants in Hawaii. They both became common and generally distributed in cane fields where they fed on young leafhoppers when this pest was at its maximum, however, due to their small size, they were not of much importance.

***Oechalia grisea*** (Burm.) (2, p. 293)

This is a large native pentatomid bug (Plate 5, Figs. 5, 7) occurring in the Hawaiian forests on all the Islands. They are particularly predacious on caterpillars occurring on the foliage of the native trees. At the time of greatest prevalence of the cane leafhopper they were found in those cane fields of higher elevation adjacent to the forests, particularly on the island of Hawaii, and fed to some extent on the leafhoppers, as well as on the cane leafroller caterpillars where these occurred.

The eggs are deposited in clusters of 10 to 20 on the surface of leaves. They are nearly spherical, flattish at top and bottom, with a circle of 9 to 11 whitish, black-tipped capitate processes around the edge at the top, the portion within this row is neatly removed as a lid in hatching. The lower half of the egg is white, the upper half, pale bronzy-green with surface finely reticulate. The life cycle has not been studied, but during the lifetime of the nymphal stages and adult many insects are eaten. They have not been observed to feed on beneficial insects.

(In his recent study of the genus *Oechalia* in Hawaii, E. P. Van Duzee, of the California Academy of Sciences, finds that what we have long considered as *grisea* is really composed of several species.)

***Chelisoches morio*** (Fab.) (3, p. 270)

***Euborellia annulipes*** (Lucas) (15)

Of several earwigs occurring in cane fields, these two cosmopolitan species (Plate 6) have been known to feed extensively on leafhoppers when the latter were abundant. They are generally predacious, feeding on whatever insects they can find in the places that they are accustomed to frequent, under trash, stones, or under bark, etc., and on cane they lurk behind the leaf sheaths and in the spindles where they could readily capture the leafhoppers. Besides eating leafhoppers, the first species was found to eat many of the egg parasites also, thus somewhat offsetting the good that it did by eating leafhoppers.

The first species is black and provided with wings, the second species is brown and wingless. They are more common in wet regions than in dry areas. The eggs of *Chelisoches morio* are deposited in masses of from 40 to 60 eggs in some secluded place, often behind the leafsheath of cane and in similar situations on other plants. They are white, broadly oval, 1 mm. long by .75 mm. in diameter increasing to twice the bulk before hatching which occurs in about 5 or 6 days. The young are actively predacious, molt 4 times at intervals of 6 to 20 days and become mature in about 50 days.

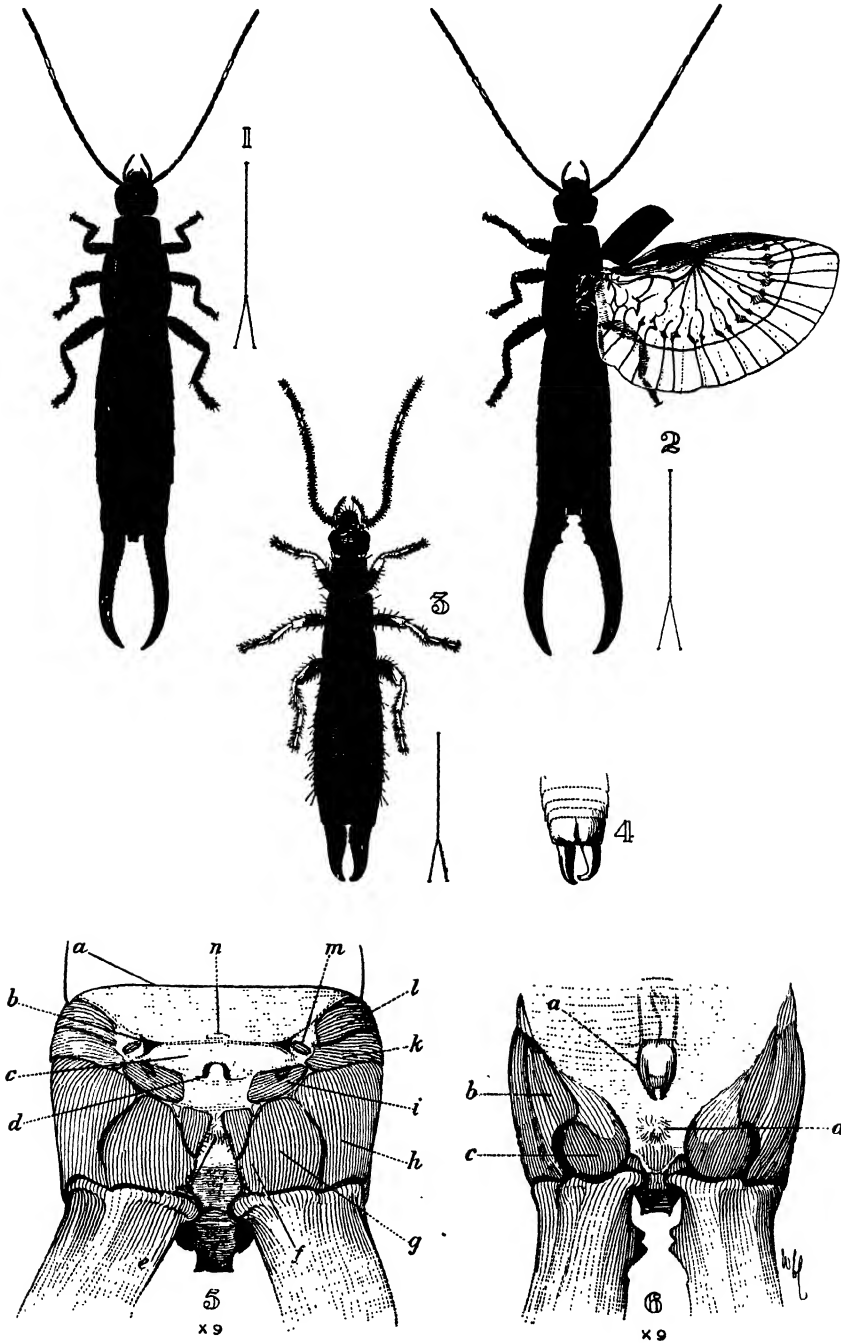


Plate 6. Earwigs.

1. *Chelisoches morio*, adult female.
2. *Chelisoches morio*, adult male.
3. *Euborellia annulipes*, adult female.
4. *Euborellia annulipes*, forceps of male.
- 5 and 6. Structure details of *C. morio* (irrelevant to the present purpose).

***Chrysopa microphya* McLachlan (16, p. 300)**

This green lacewing fly (Fig. 17) is considered an immigrant in Hawaii, but it was described from Hawaii where it has been known for many years and is not yet known elsewhere. The larvae prey especially on aphids, scale insects, etc., but in the time of maximum infestation by leafhoppers they occurred in cane fields all over the Islands and fed chiefly on young leafhoppers.

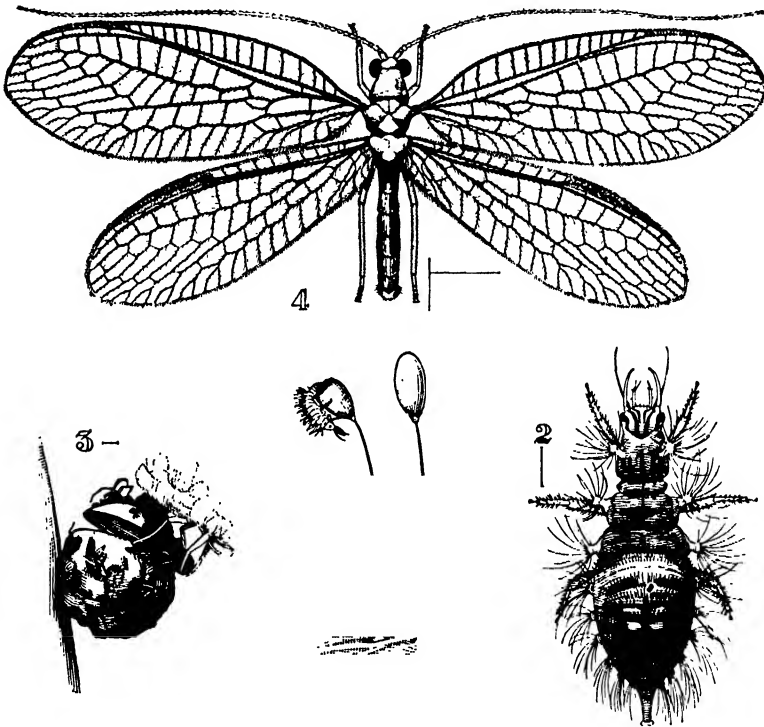


Fig. 17. Lacewing fly, *Chrysopa microphya*.

1. Egg and newly hatched larva.
2. Full-grown larva.
3. Cocoon and pupal skin.
4. Adult female.

The eggs are deposited on the under surface of a leaf, singly, or a few in a cluster, each elevated on a slender stalk several times the length of the egg. The larvae become full-fed in about 15 days, when each makes a dense, spherical, white cocoon from which the adult issues in about 10 days. As is common with many other species of *Chrysopa*, the larva of this one covers its back with the dry remains of insects from which it has sucked the fluid contents; the cocoon also is covered similarly. The cocoons are frequently parasitized by an ichneumonid, *Hemiteles tenellus* (Say). Recently another parasite has been reared from cocoons in Honolulu. It is *Isodromus axillaris* Timb. (54, p. 183) an immigrant from China. These parasites greatly check the good work of *Chrysopa*.



**Anomalochrysa deceptor** Perk. (29, p. 54)

**Anomalochrysa gayi** Perk. (29, p. 56)

**Anomalochrysa raphidioides** Perk. (29, p. 57)

**Anomalochrysa proteus** Perk. (29, p. 59)

These large species of endemic lacewing flies, occurring in the mountain forests, migrated into the upper cane fields adjacent to the forests on the island of Hawaii when their larvae found abundance of young leafhoppers for food.

The eggs of these species are laid on end directly on the surface of the leaf, not elevated on a stalk as are those of *Chrysopa microphyta*. Their larvae are usually bright colored, some green, and others pinkish or yellow, and they do not carry debris or insect remains on their backs.

The cocoons are oval to spherical, of tough silken texture, and are probably sometimes parasitized by an ichneumonid, *Hemiteles tenellus* (Say), and sometimes by a eupelmid, *Eupelmus chrysopinus* Perkins. The eggs are also sometimes attacked by *Trichogramma minutum* Riley.

Since the leafhoppers became scarce, these lacewing flies are not seen in the cane fields.

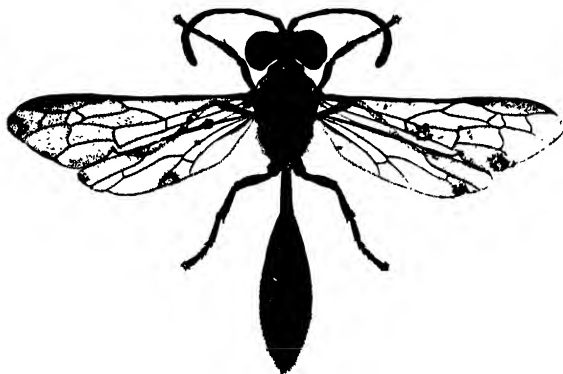


Fig. 18. *Nesomimesa hawaiiensis*, a native wasp which sometimes preys on the leafhopper.

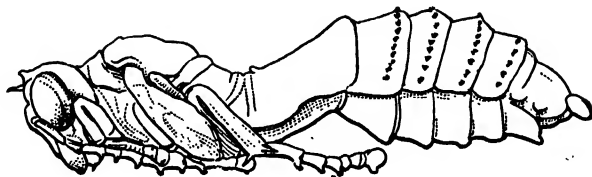


Fig. 19. *Nesomimesa hawaiiensis*, pupa.

***Nesomimesa hawaiiensis*** Perk. (28, p. 11)

This native mimesid wasp (Figs. 18-20) lives in the forests on the island of Hawaii, where it preys on native species of leafhoppers, storing them in underground nests as food for the young wasps. It was found commonly in 1918 in some cane fields at higher elevations near forested regions where its behavior was observed by Dr. Williams (61). The female wasp captured adult leafhoppers from

the cane leaves one at a time, stung and paralyzed them and carried them to store in her nest. The nest (Fig. 20) is a burrow in an exposed earth bank. The main shaft extends inwards, inclining upwards at first, and eventually turns downward and gives off several branches, sometimes as many as 18, with a cell at the end of

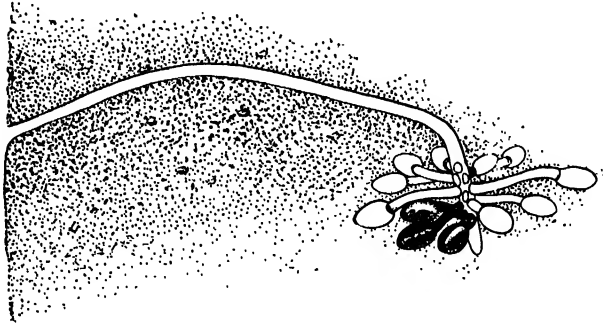


Fig. 20. *Nesomimesa hawaiiensis*, nest in the ground.

each in which are stored the paralyzed leafhoppers. In each cell 3 to 16 leafhoppers are stored, a single egg being laid on the underside of one of the leafhoppers. The larva hatches in about 2 days, and devours its store of leafhoppers in about 6 days, then spins a silken cocoon in which it transforms to the adult wasp in about a month. The usefulness of this wasp is evident, since a nest of 12 cells was found to contain 65 leafhoppers, and besides there were cocoons in 3 cells and one or two other cells in which part of the leafhoppers had already been eaten. An estimation of 100 could readily be made for such a nest, representing the activities of a single, female wasp. It is not known how many nests she may produce in her lifetime. Only a few localities were benefited by this wasp.

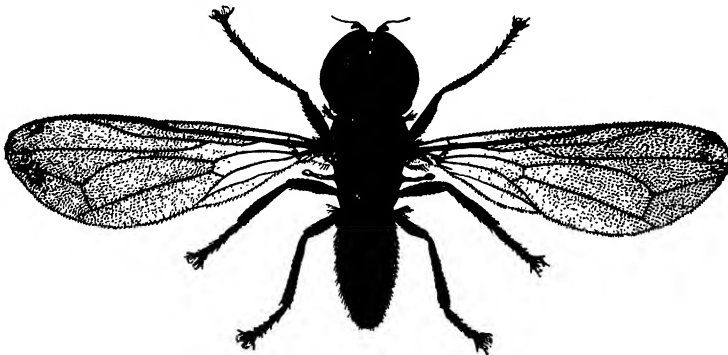


Fig. 21. *Pipunculus* sp., a native fly that parasitizes leafhoppers.

***Pipunculus juvator* Perk. (35, p. 152)**

***Pipunculus terryi* Perk. (35, p. 153)**

***Pipunculus hawaiiensis* Perk. (35, p. 155)**

These are native flies (Fig. 21) which normally parasitize native species of leafhoppers in the native forests. On the island of Hawaii, particularly, the first and

third species became very abundant in the cane fields during those years when the cane leafhopper was abundant. The second species was found in a cane field on the island of Kauai. Another species was occasionally found in cane fields on the island of Oahu.

The female fly captures a young leafhopper, preferably half-grown or less and while in flight, holding the leafhopper with her feet, oviposition takes place. The leafhopper is immediately dropped, and seems unhurt. The larva is of slow development within the young leafhopper, not reaching full growth until after the leafhopper has become mature. When parasitized by *Pipunculus*, adult leafhoppers may appear more sluggish and the abdomen is somewhat swollen. The full-grown *Pipunculus* maggot leaves the body of the leafhopper and forms its puparium on the ground in soil or beneath trash, sometimes they are found on or at the base of cane leaves. The egg and larval stage is about 40 days, and about a month is spent in the puparium, so that it is a rather long life cycle for a small fly. Only a small percentage of leafhoppers were found to be parasitized by *Pipunculus*—about 2 to 5 per cent—but in one instance 23 per cent of leafhoppers dissected were found to contain *Pipunculus* maggots (62). These flies are seldom seen in cane fields of late years when the leafhoppers are scarce.

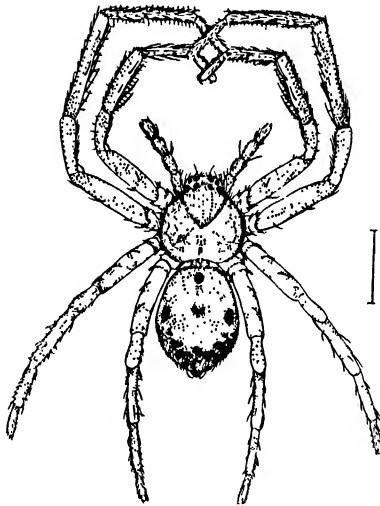


Fig. 22. *Pagiopalus atomarius*, a spider useful in destroying leafhoppers.

## Spiders

Many species of spiders were attracted to the cane fields and increased to great abundance when there were severe infestations of leafhoppers. These spiders fed extensively upon both young and adult leafhoppers, but they also preyed on other insects which were abundant on account of the leafhopper infestations, some of which were beneficial in habits, so that the spiders were not entirely useful, although they probably were in the main more beneficial than harmful.

The two species of spiders which were considered of most importance are *Pagiopalus atomarius* Simon and *Tetragnatha mandibulata* Walck. (Figs. 22-23). The former is one of the hunting spiders and does not construct a web. Its presence in cane fields where leafhoppers were numerous was made evident by the conspicuous white, flat, circular egg cocoons which were placed on the surface of the cane leaves, sometimes 2 to 6 of them contiguous in a row along a midrib, and as many as 50 having been counted on a single leaf. At one time this spider was distributed artificially by taking cane leaves with the egg cocoons attached, from fields where abundant, and transferring to other fields where they were scarce or not present.

*Tetragnatha* spiders spun webs for capturing their prey, and there is no doubt that besides the leafhoppers many of the egg parasites and other beneficial insects were caught in their webs.

*Heteropoda regia* (Fab.), another of the hunting spiders, although best known as a house spider, also frequented cane fields and although the adult spiders preyed on such large insects as roaches and grasshoppers, the young spiders could feed on the leafhoppers when abundant. Another hunting spider recorded as feeding on leafhoppers was *Adrasidca nebulosa* Simon.

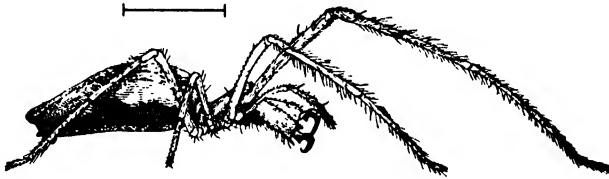


Fig. 23. *Tetragnatha mandibulata*, a spider useful in destroying leafhoppers.

Several species of jumping spiders also became numerous and utilized the leafhoppers for a part of their diet such as, *Plexippus paykulli* (Aud.), *Hasarius adansonii* (Aud.), *Bavia acriceps* (Sim.), and *Mollica microthalmus* (Koch.).

## Enemies of Spiders

There are several species of wasps which store up spiders in the cells of their nests for the wasp larvae to feed on. The best known is the common mud-dauber *Sceliphron caementarium* (Drury). Others in Hawaii are *Pison hospes* Sm., *Pison iridipennis* Sm., *Pison argentatum* Schuck., *Trypoxylon bicolor* Sm., *Trypoxylon philippinensis* Ashm. and *Anoplius luctuosus* (Cress.). The larvae of two species of ichneumonids feed on spider eggs in their silken egg cocoons—*Arachnoleter swaseyi* Cush. and *Tromatobia rufopectus* (Cress.). The larvae of two species of flies of the family Drosophilidae feed similarly on certain spider eggs—*Titanochaeta ichneumon* Knab and an undetermined species.

Various cane field spiders are attacked by one or more of these enemies listed and thus their efficiency lessened. *Arachnoleter swaseyi* and *Tromatobia rufopectus* are in turn attacked by a parasite, *Pleurotropis wilderi* (How.), which issues in numbers from their cocoons. A parasite, *Eupelmus melanotarsus* Perkins, similarly issues from the puparia of the fly *Titanochaeta ichneumon*, mentioned above.

## FUNGUS DISEASES OF THE LEAFHOPPER (39, pp. 54-55)

**Entomophthora sp.****Sporotrichium sp.****Cordyceps**

In the regions of greatest humidity there were times or seasons when one or more of these fungus diseases was particularly effective in killing off almost the entire leafhopper population in severe infestations. Probably the *Entomophthora* was the most effective and it was more prevalent on the plantations of the wetter districts on the island of Hawaii than elsewhere. The whitish, fungus-covered, dead leafhoppers would be seen in large numbers stuck to the surface of the cane leaves. This was not of regular occurrence but was very effective at times. Of late years with the scarcity of leafhoppers in cane fields, fungicized specimens are seldom seen.

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- (63) ———, 1931. Handbook of the insects and other invertebrates of Hawaiian sugar cane fields. 389 pp., numerous illustrations.

## APPENDIX A

### DISTRIBUTION OF LEAFHOPPERS OF THE GENUS *Perkinsiella*, CHIEFLY SUGAR CANE INFESTING INSECTS

- (1) *P. saccharicida* Kirkaldy. *The Entomologist*, 36, p. 179, 1903. Hawaii, Australia, Federated Malay States, Formosa, South China; and Natal, Mauritius, Fiji (one specimen each).
- (2) *P. vastatrix* (Breddin). *Deutsch. Ent. Zeit.*, p. 107, 1896. Java, West Borneo, Amboina, Ceram, Papua, Philippines, Federated Malay States.

- (3) *P. graminicida* Kirkaldy. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 1, Pt. 9, p. 406, 1906. Queensland.
  - (4) *P. vitiensis* Kirkaldy. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 1, Pt. 9, p. 406, 1906. Fiji, Samoa, Savage Id. or Niue.
  - (5) *P. sinensis* Kirkaldy. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 3, p. 138, 1907. China, West Borneo, Japan.
  - (6) *P. pallidula* Muir. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 9, p. 6, 1910. Borneo.
  - (7) *P. rattlei* Muir. Expt. Sta., H.S.P.A., Ent. Ser. Bul. 9, p. 6, 1910. Papua, New Caledonia.
  - (8) *P. bicoloris* Muir. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 9, p. 7, 1910. Papua.
  - (9) *P. lalokensis* Muir. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 9, p. 9, 1910. Papua.
  - (10) *P. variegata* Muir. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 9, p. 8, 1910. Papua.
  - (11) *P. papuensis* Muir. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 9, p. 9, 1910. Papua.
  - (12) *P. amboinensis* Muir. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 9, p. 10, 1910. Amboina.  
(*P. fuscifrons* Muir. Exp. Sta., H.S.P.A., Ent. Ser. Bul. 9, p. 11, 1910. Amboina. This was later referred to *Dicranotropis*.)
  - (13) *P. insignis* (Distant). Ann. Mag. Nat. Hist., (8), IX, p. 190, 1912. India, West Africa (1 spec.).
  - (14) *P. facialis* (Distant). Ann. Mag. Nat. Hist., (8), IX, p. 191, 1912. India.
  - (15) *P. thompsoni* Muir. Proc. Haw. Ent. Soc. II, No. 5, p. 240, 1913. Guam.
  - (16) *P. bakeri* Muir. Philippine Journ. Sci., XI, p. 379, 1916. Philippines.
  - (17) *P. saccharivora* Muir. Philippine Journ. Sci., XI, p. 379, 1916. Philippines.
  - (18) *P. lineata* Muir. Philippine Journ. Sci., XI, p. 380, 1916. Philippines.
  - (19) *P. fuscipennis* Muir. Philippine Journ. Sci., XI, p. 380, 1916. Philippines.
  - (20) *P. pseudosinensis* Muir. Philippine Journ. Sci., XI, p. 381, 1916. Philippines.
  - (21) *P. manilae* Muir. Proc. Haw. Ent. Soc., III, p. 324, 1917. Philippines.
  - (22) *P. vitalisi* Muir. Ent. Mo. Mag., 61, p. 222, fig. 1, b., 1925. Indo-China.
  - (23) *Perkinsiella* sp., (unidentified, near *bakeri*). West Africa.
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## The Day-Degree in Mauritius

For the last few years we have been urging the use of the day-degree as a measure of effective warmth as it relates to cane growth.\* It is, therefore, with great interest that we give here a brief review of an able paper by Pierre Halais of Mauritius† on the use of the day-degree.

Mr. Halais' contribution to the subject is that he suggests correction factors to the day-degree to take care of variations in the moisture content of the soil and the age of the plant. He contends that under Mauritius conditions cane growth does not take place below 20 per cent of soil moisture and that moisture above 40 per cent is of no use to the plant. He, therefore, assigns a value of 1 (100 per cent) to the upper limit of 40 per cent and 0 to the lower limit of 20 per cent of soil moisture. He

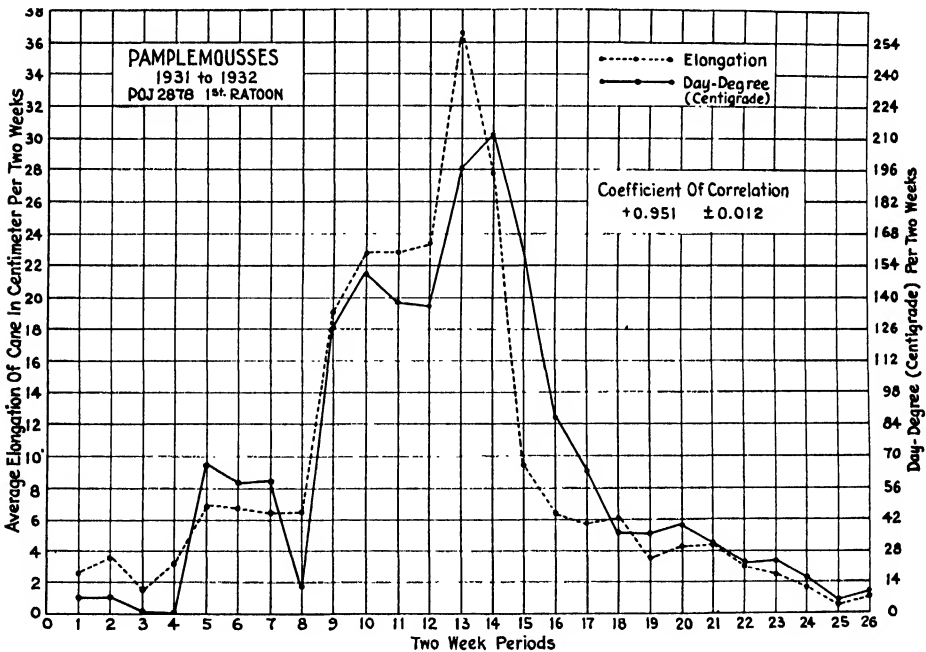


Fig. 1

uses linear interpolation between these limits, i. e., 30 per cent moisture is considered to have a value of 0.5, 25 per cent moisture a value of 0.25, and so on. The day-degree is then multiplied by these effective values. (We should note that his reasoning here is not in harmony with the experience of our irrigation experts.)

\* See Agee, H. P., and Das, U. K., 1933. Proc. Ass'n Haw'n Sugar Tech., pp. 45-48, and Das, U. K., 1933. The Hawaiian Planters' Record, Vol. XXXVII, pp. 32 and 174.

† Un Nouvel Indice de Climatologie Agricole, La Revue Agricole, Maurice, No. 80, March-April, 1935.

He corrects for the age effect by assigning a value of 1 to the first-year cane before tasseling and 0.5 to the cane after tasseling. The day-degree is again adjusted accordingly.

In regard to the day-degree itself, he assumes that its value increases somewhat as the temperature rises above certain limits.

Employing the above corrections he finds a correlation of  $+0.95$  between actual cane growth and the adjusted day-degree. A typical chart is reproduced below from his articles (Graph III).

Our purpose in bringing this article to the notice of our readers is not to recommend his methods, some of which are open to question, but to indicate that here lies a fruitful field of research for the plantation agriculturist—one that is bound to yield results of great practical value.

U. K. D.

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## Sugar Prices

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96° CENTRIFUGALS FOR THE PERIOD  
SEPTEMBER 16, 1935, TO DECEMBER 7, 1935.

Date	Per Pound	Per Ton	Remarks
Sept. 16, 1935.....	3.47¢	\$69.40	Cubas.
“ 17.....	3.45	69.00	Philippines.
“ 23.....	3.54	70.80	Cubas, 3.55, 3.57; Philippines, 3.50, 3.55.
“ 27.....	3.59	71.80	Cubas.
Oct. 2.....	3.65	73.00	Cubas.
“ 4.....	3.68	73.60	Cubas.
“ 14.....	3.60	72.00	Philippines.
Nov. 1.....	3.50	70.00	Cubas.
“ 8.....	3.40	68.00	Puerto Ricos.
Dec. 4.....	3.10	62.00	Cubas.
“ 6.....	3.07	61.40	Cubas.
“ 7.....	3.10	62.00	Cubas.

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3-7-39

# THE HAWAIIAN PLANTERS' RECORD

Vol. XL

SECOND QUARTER, 1936

No. 2

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*A quarterly paper devoted to the sugar interests of Hawaii and issued by the Experiment Station for circulation among the plantations of the Hawaiian Sugar Planters' Association.*

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## **In This Issue:**

### *A Suggested Scheme of Irrigation Control Using the Day-Degree System:*

How to regulate irrigation from season to season has always been a problem. This article suggests that weather observations may well form the basis of a satisfactory control scheme.

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### *Studies in Experimental Technique:*

The search for a more nearly perfect experimental technique for field tests continues. Arguments for and against the practice of discarding border rows are usually decided by the practical consideration of whether or not these outer rows can be eliminated at harvest without the introduction of a more serious error caused by attempts to separate heavy tonnage cane from the inner rows. Arguments for and against a random vs. a regular series arrangement of the plots on the test area have usually been decided in favor of the experimenter's preference.

We now offer a balanced block arrangement of treatments that appears to have considerable merit, in that it definitely reduces the amount of border effect between treatments, and it makes allowance for any definitely natural "fertility slope" that may exist within the test area. Furthermore, the more accurate yield data secured from this arrangement are suitable to statistical interpretation by the analysis of variance, which greatly reduces the experimental error and makes for a more valid estimate of the effect produced by the treatments included in the experiment.

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### *POJ 2878 Cane in the Factory:*

Comments are given on POJ 2878 cane from the factory standpoint covering the crops of 1933, 1934 and 1935. These crops represent the periods in which this variety was ground in any appreciable quantity. This cane has caused clarification difficulties at various factories from time to time. A light sulfitation of mixed juice at a few factories having acute difficulties has solved the problem. The manufacture of raw sugar and its refining qualities appear to be no different than

that from other varieties, providing the clarification has been satisfactory. There is some evidence that the steam generating quality of the bagasse is poorer. The fiber content of this cane and, therefore, the percentage of bagasse appear to be lower than that of most of our other canes. These qualities may possibly be accounted for in that this cane matures in several months less time than the standard varieties.

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#### *Utilization of Molasses:*

The beef cattle industry in these Islands has been well developed as to the ranges and the breeding of the cattle. The full hereditary possibilities of these well-bred animals in quality beef production is not realized because they are not "finished" on concentrated feeds. Practically all of the good beef is now imported from the Mainland.

The feeding value of molasses and bagasse is discussed, and suggestions made for the development of beef production in the Islands.

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#### *The Third International Congress of Soil Science:*

A discussion is presented which bears upon the proceedings of the Congress and upon matters relevant to soil chemistry as they developed at informal gatherings of delegates.

About four hundred persons from sixty countries attended the sessions of the Congress which were held at Oxford University, England, in the late summer of 1935.

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#### *Absorption of Essential Chemical Elements by Segregated Roots of Sugar Cane:*

Normal cane growth is reported in experiments wherein nine essential elements were separately absorbed by nine isolated roots. The dissimilarity of the roots in the several solutions, particularly the superior root development in the solution containing calcium, is a matter of theoretical interest. Experiments are cited which demonstrate that a part of the growth response of Sudan grass in certain soils to large applications of superphosphate is due to the calcium constituents of the fertilizer. Both phosphorus and calcium augment the resistance of cane to *Pythium* root rot.

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#### *Cane Growth Studies:*

Numerous studies have been made of the measurable effects of different soil types, different cane varieties, and different levels of fertilization upon cane and sugar yields and juice quality. To these studies we now add another issue which offers rather convincing evidence that even the relatively small differences in climate that exist in our cultivated sugar cane areas may dominate these more obvious factors of soil, variety and fertilizer. This should arouse further interest in efforts to regulate field procedures and practices so as to take the fullest advantage of the climate that is given us.

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*Rat Control Investigations at The Lihue Plantation Company, Ltd.:*

Rat damage to sugar cane on the island of Kauai is probably greater than on any other island in the Territory. The extent of the problem at The Lihue Plantation Company, Ltd., is given. The large and voracious species of rat, *Rattus norvegicus*, is apparently responsible for all of the damage and is the most difficult of all of the species in Hawaii to control. The standard poison bait used during the past seven or eight years on most of the plantations has not given satisfactory results on Kauai, Lanai, and, to some extent, on the other islands. Recent studies have resulted in an improved bait which contains a higher concentration of thallium sulphate per unit of grain. Rolled barley or rolled oats has also been found to be much superior to whole wheat as bait material. Thomas G. Eckart of The Lihue Plantation Company, Ltd., has gone a step further in the improvement of baits by adding certain aromatic vegetable oils separately to the grain and paper wrapper. The results of many field tests are given which show definite advances in rodent control through the use of alluring oils in particular ways.

*Yams:*

Today the question of diversified agriculture is a live subject with the plantations. In this article a food crop that plays an important part in the domestic economy of many tropical countries is presented for consideration in this connection.

*Standard Methods for Measuring Primary and Total Combustion Volumes in Mill Boilers, Also for Measuring Mill Roller Openings:*

The methods adopted as official by the Association of Hawaiian Sugar Technologists and used by the factories in preparing schedules for the Annual Synopsis of Mill Data are presented.



## A Suggested Scheme of Irrigation Control Using the Day-Degree System

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By U. K. DAS

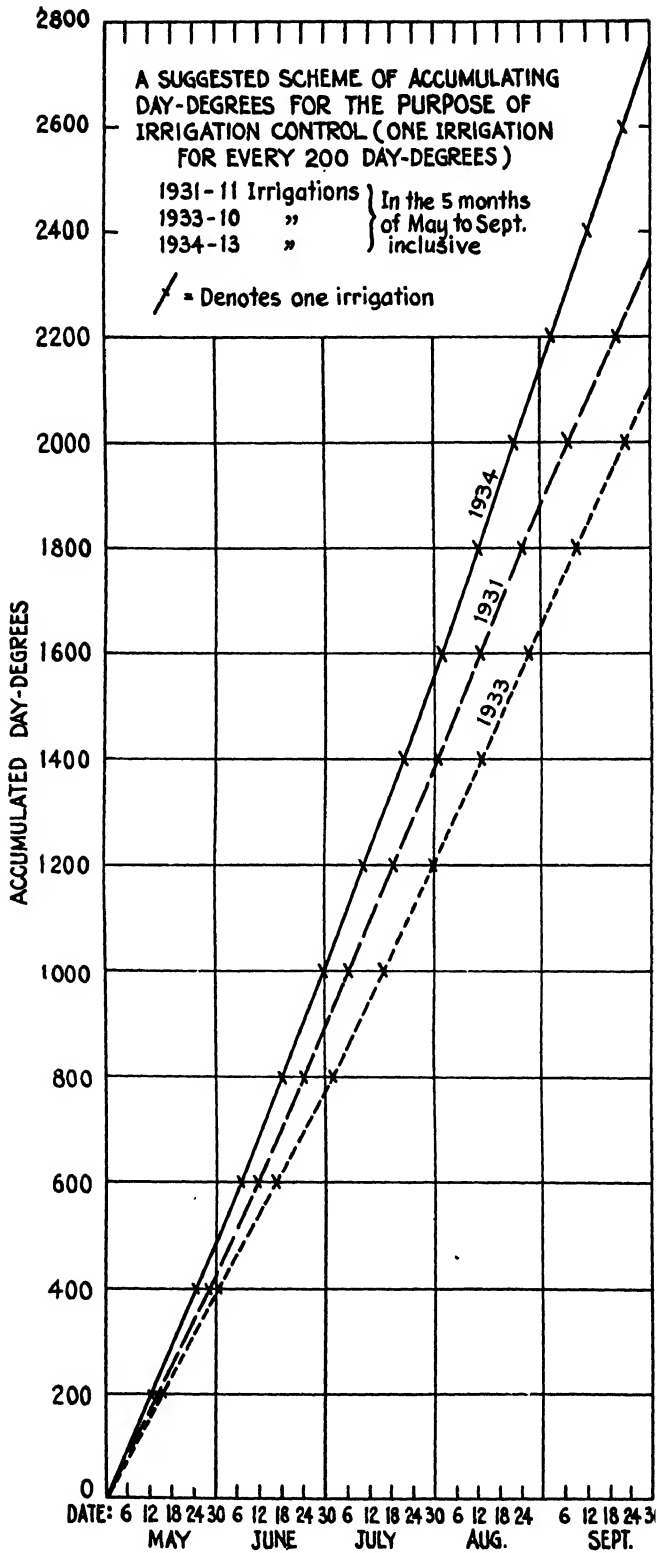
One of the chief objectives of irrigation control is to regulate the distribution of water from season to season so as to provide for the changing demands of the cane plant. In most places such control depends on the observations and the past experience of the irrigation overseer. In recent years, however, some plantations have been developing a scheme of irrigation control based on actual soil moisture determinations. It is the purpose of this paper to indicate that in our day-degree system we may have a satisfactory and convenient guide to the seasonal demands of the cane plant.

To start with we shall assume that the seasonal differences in the water requirements of the cane plant are due, largely at any rate, to the differences in the conditions of light and heat. If we could measure the amount of light and heat that affect cane growth, then we could regulate our water distribution accordingly. In our past studies it has been shown that in our "day-degree" we have a very satisfactory index of the conditions of warmth (and probably, light associated with it). Would it not, then, be reasonable to think that we may obtain a satisfactory control of irrigation if we always maintain a definite relation between the amount of water and the growth-promoting factors as measured by the day-degree?

How can we do that in practice? If we have growth measurement data accumulated over a series of years, then, from these data we can select a group of months which have been excellent for growth. We may now reasonably assume that in these months there was sufficient water applied to the cane. Therefore, from the relevant data we can determine the relation between the day-degrees and the percentage of area irrigated in those months. In the case of one plantation, the data of which we were permitted to study, we found the general relation to be about one round of irrigation or hundred per cent of the area irrigated per two hundred day-degrees. (Where cane growth data are lacking such a relationship as the above may be tentatively based on personal experience.)

Having established, let us say, that two hundred day-degrees require one irrigation, we can accumulate the day-degrees from day to day and order one round of irrigation whenever the accumulating sum, since the last irrigation, reaches the two hundred mark. In fact we shall be able to foretell the irrigation needs a few days ahead by studying the trend of the curves if we are plotting the day-degrees on a graph. The accompanying figure shows the progress of the day-degrees on one of our irrigated plantations for the years 1931, 1933 and 1934, and the irrigation rounds that would be called for if we were to give one round for every two hundred day-degrees. Whereas 1933 required only ten irrigations from May to September, 1931 required eleven, and 1934 thirteen for the same period of time. In other words, if we were to maintain a constant relationship between water and effective warmth, then we would have had to apply thirty per cent more water in 1934 than in 1933.





A scheme like the above should be looked upon as we do a "basic fertilizer policy;" in other words, deviations from the scheme will be in order whenever experience indicates such to be necessary. Thus we may find that in very young cane we need to apply less than one round for X day-degrees (X may be two hundred or any other value established for the locality), whereas in old cane more than one round is required. Again, similar adjustments may have to be made in the case of heavy and of porous soils—the basic scheme being to apply on the average the same amount of water for equal amounts of effective warmth.

How to adjust for rainfall? In the arid or semi-arid districts like Waianae or Ewa an inch of rainfall may be considered as equal to one-tenth of an irrigation (i. e., ten per cent of the area irrigated). This value may be somewhat different in different places. However, once we have decided from experimental or observational data what value to give to rainfall, we can take that into account in determining when to apply the next irrigation. In general it may be advisable to underestimate rather than overestimate the value of scattered showers.

The above scheme is offered only as a suggestion. The method appears so simple and yet so full of possibilities that it may be worthwhile to set up experiments comparing the plantation practice of irrigation control with the method of control here suggested. It may be advisable to try several ratios—say one round for every 150, 200, or 250 day-degrees, before deciding which is the best under the particular conditions. Even if the scheme be found on experimentation to lack the precision of some of the more elaborate methods, it may provide a control satisfactory enough for practical purposes.

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## Studies in Experimental Technique

### THE BALANCED BLOCK ARRANGEMENT OF TREATMENTS

By R. J. BORDEN

The statistician who is not also an agronomist usually insists that a valid estimate of the error in a field experiment can only be obtained when the replicates are distributed *at random* on the test grounds. He is likely to look askance at any plot arrangement that appears, on paper, to be regular or systematic. Some agronomists, however, are inclined to feel that a balanced arrangement that is based somewhat on the order of Beaven's "Half-drill strip" plan, i. e., A B B A A B, etc., or an intelligently planned placement of the treatments that are to be compared, may not be entirely wrong, and may in fact have features that are extremely worthwhile.

We fully recognize the value of the probable error concept, in securing the valid estimate of error we desire in order to demonstrate that some differential treatment which we have applied has affected the yields, and to measure the extent of such treatment effect. We recognize that this probable error concept depends upon unbiased or random sampling and a normal frequency distribution, and that the probable error value which is calculated for the relatively few replicates that we can install in field tests is only an estimate of the true probable error. We have no objection to random arrangements as usually advocated but we are not entirely convinced that they are necessary, and we have a feeling that a balanced, definitely planned arrangement may have some real advantage.

Our studies of many hundred field experiments with sugar cane, where the replicated plots have been laid down in a regular series arrangement, as A B C D A B C D A B etc., have not demonstrated the existence of any continuous fertility gradient which has significantly affected the yields obtained and caused an erroneous interpretation of the treatment effect. Short gradients of fertility have occasionally been found within the test areas, and so we have sometimes laid down the replicated plots in two columns of plots, and have reversed the series arrangement in these adjacent columns so as to take care of this possible occurrence, e. g.,

A B C D A B C D A etc.

D C B A D C B A D etc.

It seems quite evident that the larger part of our sugar cane areas are characterized by "spotty" fertility differences rather than by any regular "fertility slope." We have harvested many so-called "blank tests" wherein all plots had received a similar treatment, and the nature of the yields secured has convinced us that fertility differences exist purely at random in our cane field. Thus it is our belief that the randomness which is desired for a true estimate of the experimental error is actually obtained when the first plot of our experiment is assigned its location in the field, and that the spotty nature of our soil fertility gives still further randomness to the plots which follow. Thus what may appear to be a systematic or regular arrange-

ment of plots on paper, is actually a random arrangement on the soil, and no additional efforts to randomize their location can afford anything more than personal satisfaction. If this view is sound then we can proceed with an intelligent placement of the treatments that are to be compared.

The majority of the field experiments which are concerned with problems of fertilization for sugar cane, are designed to determine the optimum amount of nitrogen, of phosphoric acid, or of potash, for areas that are found to be deficient in these plant foods. Hence in these "amounts" tests, we are interested not only in determining that there has been a treatment effect on the yields, but also in determining the significance that can be attached to the amount of yield difference which is the effect of each successive increment of the plant food supplied. For instance, we may be interested in ascertaining whether an application of 150 pounds of nitrogen will be likely to give us a real sugar-tonnage gain over 100 pounds, and whether 200 pounds will give us a still further gain over 150 pounds; we shall not be particularly concerned with the gain of 200 pounds over 100 pounds, unless it is a definite gain over 150 pounds too. Thus we may plan an arrangement of treatments in such a way that those comparisons we most desire are made on closely adjacent plots which our "blank test" data indicate are more apt to be alike than plots which are separated.

The real value of a definitely planned assignment of plots comes from its possibilities in reducing the "border effect" between adjacent plots carrying different treatments. This border effect has been recognized and variously dealt with by all who have carried on field tests, and it is known that its magnitude increases as the adjacent treatments are made more widely different. Hence, if the adjacent plots in a field test are given the minimum possible difference in treatment, we shall go a long way towards reducing the border effect to a minimum.

Our attempts to eliminate border effect in experiments with sugar cane by cutting out the outer row of adjacent plots and discarding this cane, are quite apt to introduce even greater errors than we are trying to get rid of. In experiments which have a heavy tangled mass of cane stalks at harvest, the difficulty with making a single clean separation of cane between plots is well known, and if two separations must be made in order to discard the outside row of each plot, the error of separation is apt to be doubled. Thus any plan which will reduce the border effect to a point where it will be unnecessary to cut out and discard the cane of the border rows needs our thoughtful consideration.

On the assumption that our reasoning is sound, we have proposed and encouraged the use of a balanced block arrangement of treatments for field experiments that are concerned with issues of fertilization. The experimental area is divided into blocks, and each block is of sufficient size to include a single plot of each treatment. The blocks need not all be of the same size, but it is desirable to have the plots in a single block as near alike as possible in number and length of lines, topography, exposure, soil depth, drainage, stand of cane, etc. The relative position of the blocks with respect to one another is immaterial, and where the topography is irregular, they may be separated by areas of "crop cane." Soil and crop *uniformity within any one block* are the primary considerations. Hence we may make use of a plan that does not necessitate our having an area of several acres of uniform soil type and topography, and it becomes much simpler to find acceptable places for our experiments.

## KAHUKU PLANTATION CO.

EXP. 56AN, 1936 CROP

POJ 2878 - First ratoons in thirty  
9-line plots in ten Blocks.

Yields as T.S.A. @ 15 months.

Plot No. Treatment T.S.A.	30 A 3.8	29 X 5.0	28 B 4.8	27 B 4.8	26 X 5.7	25 A 4.7	24 A 5.2	23 X 6.7	22 B 6.2
Plot No. Treatment T.S.A.	21 B 6.0	20 X 6.4	19 A 5.7	18 A 4.9	17 X 5.6	16 B 6.0	15 B 6.5	14 X 6.0	13 A 6.3
Plot No. Treatment T.S.A.	12 B 7.4	11 X 6.9	10 A 6.3	9 A 5.9	8 X 6.4	7 B 6.1			
Plot No. Treatment T.S.A.	6 A 5.5	5 X 6.4	4 B 6.1	3 B 5.4	2 X 6.2	1 A Lost * Est. @ 5.1			

\* Estimated by  
method of  
Allan and Wishart

A deliberate plan is made on paper, and each treatment is assigned a place in each block, the objective being to place the treatments in such a way that the minimum possible border effect will ensue. This idea is carried still farther by reversing the order of plots in the adjacent blocks, so that there will be no border effect between many of the plots on the test area. This reversal of plot assignments in adjoining blocks also has the advantage of providing a balanced arrangement in case there should be a pronounced fertility slope.

After the crop has been grown and harvested, the yields that are obtained may be examined by Fisher's "Analysis of Variance" in order to determine whether there has been a definite effect of treatment, and if there has been, then to measure the significance of the amounts of difference that were found between the differential treatments given.

We present in the accompanying figure a plan of one of an "Amounts of Nitrogen" experiment, laid out according to our balanced block arrangement, which has now been harvested. The features which we have discussed are apparent.

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DATA TO ACCOMPANY ILLUSTRATION ON PAGE 115

Plan of Fertilization—(Pounds Nitrogen Per Acre)—					Results:
Plots	Dec.	Feb.	April	Total	Avg. Yield T. S. A.
A	72	28	0	100	5.34
X	72	78	0	150	6.13
B	72	78	50	200	5.93

An examination of these yield data, by the analysis of variance, quite clearly shows that there has been an effect of treatment, and indicates that an amount of difference between the treatment averages which is greater than .3 ton of sugar would be significant. Thus it is apparent that the experiment has shown a reliable gain for an application of 150 pounds of nitrogen over 100 pounds but that there was no further gain for the 200 pounds application.

## POJ 2878 Cane in the Factory

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By W. L. McCLEERY

In the Experiment Station Director's Monthly Report issued December 10, 1933, the writer presented a short summary of the qualities of POJ 2878 cane from the factory standpoint. The crop of 1933 had just been completed and the Synopsis data for that year showed that this variety comprised over one per cent of the crop harvested at nine factories.

Clarification difficulties had been encountered during 1933 at several factories when grinding POJ 2878 cane and considerable concern was felt in various quarters as to the probable refining qualities of the sugar produced from this variety. Early in the 1934 grinding season, requests were sent to the plantations that were to harvest POJ 2878 cane, asking that samples of sugars be sent us from POJ 2878 and from other (standard) varieties. The request for these samples was made in order that we might obtain information on the filtrability and color characteristics of sugar from POJ 2878 in comparison with those of sugars from the standard varieties and for possible further study.

Later in the 1934 season, as soon as the few factories having any considerable amounts of this cane began grinding it unmixed with other canes, it was apparent that one of the major factors influencing the refining quality would be the quality of the clarification and if the clarification were satisfactory, the sugar refining qualities would follow closely those of other varieties. This has since been fully confirmed. We had long recognized that there was a marked correlation between the clarity of the juice sent to the evaporators and the characteristics of filtrability, original color and crystal color of the sugar.

The clarification difficulties with POJ 2878 cane were much greater than were expected when this variety was originally planted on a field scale, although several factories have had no particular difficulty with it. Other varieties such as Uba, D 1135 and at times H 109, had also given trouble in clarification. It was soon realized that some modification in clarification procedure would have to be practiced to meet the difficulty and allow the factories to operate at normal capacity.

Of the many modifications in clarification procedure that have been tried, the addition of ammos-phos A has given the best results with many moderately refractory juices, particularly those with a low  $P_2O_5$  content. With the more difficult juices, sulfitation has proven to be a very satisfactory solution of the problem.

POJ 2878 accounted for 8.3 per cent of the 1935 tonnage of cane harvested. In 1934 it was 3.2 per cent. At the close of the 1935 crop, seven of the factories that have ground this cane had installed equipment for mixed juice sulfitation, largely as an insurance against clarification difficulties. Two others were preparing to install similar equipment for 1936. The few remaining factories having fair quantities of POJ 2878 have not been seriously affected.



The average crystal color of raw sugar from all factories was more satisfactory in 1935 than in 1934 and also better than in any previous year. The filtrability of the Crockett receipts for 1935 was 3 points better than in 1934 and practically equal to the 1933 and the 1932 averages. The tendency toward better filtration rates is especially gratifying as it is against the expected trend due to the recent gradual reductions in sugar pol.

The subject of clarification is still a major project, with our efforts now directed principally toward modifications which will aid in securing larger elimination of impurities than is obtained by the usual procedure.

The milling quality of POJ 2878 would appear to be in line with that summarized in the report of 1933. The data were largely from Waimanalo Sugar Company's factory as this plantation began spreading POJ 2878 soon after it became available. Extracts from this report are given in the following paragraphs:

**Milling:** POJ 2878 cane is classed as long-jointed, medium-heavy stalk, self-stripping, with comparatively hard rind. It appears to mill well, that is, feeds into the mills without difficulty under conditions of close settings and heavy pressures. Waimanalo reports that the extraction is slightly higher with this cane than with II 109.

**Bagasse Burning Quality:** The interior portions of the joints are more pithy than in other canes and the steam generating quality of the bagasse seems to be poorer due to its lightness and fineness. This necessitates a more careful distribution of air through the furnace tuyeres and grates, and close supervision for keeping the grates sufficiently well covered to prevent air holes, but not too thick for rapid combustion.

The mechanical structure of the bagasse from various varieties of cane has appeared to have more influence on steaming quality than moderate changes in fiber content of the cane.

Waimanalo had considerable difficulty through steam and fuel shortage last year when burning any considerable quantity of POJ 2878. The fiber this year was also low at times. In addition to more careful work in the fireroom, they have had to resort to vapor heating in the boiling house to reduce steam consumption.

The thermal value of dry bagasse from POJ 2878 at Waimanalo has been found to be on a par with other varieties at 8330 B.t.u. A number of tests, also made at the Station in 1928, of bagasse classed as good burning and poor burning revealed a minimum of 8037 B.t.u. and a maximum of 8363. A complete survey of all the major varieties in 1912 gave 8089 as the minimum and 8344 maximum.

From present indications the per cent fiber in POJ 2878 appears to be practically the same as in other canes when the juices are of good purity, but with low purities the fiber is low. If purity is judged as a sign of cane maturity, it can perhaps be expected that with mature cane the fiber content will be normal. The tabulated results from the Waimanalo reports indicate a clear relationship between purity and fiber.

**Boiling:** Present information does not indicate that POJ 2878 juices (after passing clarification) act differently in boiling-house practices than other juices. There is no direct evidence that the quality of commercial sugar is affected nor the purity of final molasses.

**Waimanalo Data:** Certain laboratory data from the 14 Weekly Mill Reports are tabulated below in which 63 per cent to 100 per cent of the cane ground was POJ 2878. The weeks are arranged in the order of descending purity of first expressed juice, which indicates a close relationship between purity and fiber content and also, as would be expected, the pol in cane.

Crop Week Nos.	First Expressed Juice Purity	Per Cent Fiber	Per Cent Cane Pol
26	84.9	13.41	13.44
27	84.1	13.38	14.01
32	82.9	13.05	12.69
39	82.3	12.13	14.89
41	82.2	13.64	11.77
28	81.7	13.20	14.15
34	81.3	12.23	13.23
31	81.2	13.09	12.17
38	81.2	12.09	13.92
33	81.1	12.98	11.71
40	79.0	12.11	11.34
36	78.7	11.65	13.44
37	78.0	11.90	12.82
35	76.7	11.45	13.15
Average 1st 7	82.77	13.01	13.45
Average 2nd 7	79.44	12.18	12.65

Since writing the 1933 report, POJ 2878 cane has become the principal variety at Waimanalo owing to its superior yield on that plantation in sugar per acre month. The following figures for four crops show two crops of POJ 2878 and two of H 109 when grinding an average of about 80 per cent of these varieties. The figures are taken from Synopsis data.

Crops Avg'd	Per Cent		Per Cent Fiber	Per Cent Pol	Brix 1st Juice	Purity 1st Juice	Cane Ratio
	POJ 2878	Per Cent H 109					
1934-1935	82	None	12.08	11.12	17.05	79.7	10.39
1930-1931	None	79	13.45	11.43	17.32	82.6	9.94

It appears that there is a marked tendency toward lower cane fiber and juice purity and a probable tendency toward lower cane pol and juice density with POJ 2878 than with H 109, at least under Waimanalo conditions. This is perhaps to be expected since the period of growth for POJ 2878 is several months shorter.

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## Utilization of Molasses

### THE USE OF CANE BY-PRODUCTS IN FATTENING BEEF CATTLE

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By A. R. LAMB

There are some forty-one cattle ranches in the Territory of Hawaii which carry over 100,000 cattle, of which well over 90 per cent are beef cattle. In addition to these larger ranches there is about an equal number of small ranches. The total number of beef cattle in the Territory was estimated in 1928 as over 157,000. This statistical information, as well as some similar information given below, is the result of a careful survey by Prof. L. A. Henke of the University of Hawaii, the results of which were published in 1929.

According to his survey, the total area of these forty-one ranches is 1,115,200 acres. This is 32 per cent of the total area of the Territory and six times as great as the area planted to sugar cane. Of course, a great deal of this is waste land, with sparse vegetation, but most of it takes its turn, according to season and rainfall, in supporting animals which graze upon it.

A few of the ranches have improved the land appreciably by sowing grasses of better quality which are adapted to the various areas. A few other ranches are fortunate enough by reason of well distributed and sufficient rainfall to have areas which grow plenty of nutritious forage. These few ranges grow animals to maturity efficiently enough so that they fill out well and put on some fat, especially if finished on the best pastures, fenced in and supplied with water.

#### THE LOCAL MARKET FOR BEEF

Such cattle make fairly good beef, although not as good as that which receives its final fattening on good legume pasture or "concentrate" feeds such as corn, oil meal, etc. This range-fattened beef is marketed locally and in Honolulu. In Honolulu it is apparently marketed mostly at a much cheaper price than well-finished (well-fattened) beef from the Mainland. This Island beef seems to have inherent quality due to good type in the animal on the hoof, but it is tough and somewhat lacking in flavor because it is not "finished" by means of a final fattening period. Such beef has been selling in a good Chinese market at 9 to 10 cents less per pound than the same cuts of mainland beef at Piggly Wiggly Stores.

A large section of the customers on all these Islands buys the cheaper grades of meat and prefers such beef because it is not fat. All of the better class of the trade, however, prefer well-fattened beef, even though some of the outside layer of fat is wasted, because of the effect of the fattening on texture and flavor. It is said that some of the range beef is sold to the Navy, but it does not generally come within the government specifications as to quality and finish, and the Army now brings more than 95 per cent of its beef from the Mainland.

Some idea of the size of the Island market for good beef may be obtained from figures published by the Honolulu Advertiser, presumably from information furnished by the U. S. Customs Service. The amount of fresh chilled or frozen beef

and veal imported in 1932-33 was 3,242,000 pounds, wholesale value, \$382,000; and for 1933-34, 3,835,000 pounds, valued at \$400,000. Of this amount, about 1,200,000 pounds were imported for the Army.

This is only about 18-20 per cent of the total amount of beef consumed here, since the total beef killed in the Territory in 1934 is estimated at 33,000 animals, yielding 16,000,000 pounds of dressed beef. (Honolulu Chamber of Commerce.) In this connection it must be remembered that the greater part of the beef on any market is always inferior in quality and finish, as in other commodities. A certain fraction of the trade, however, greater or less under varying economic conditions, demands good or choice beef. In this Territory this fraction is probably rather high because of the large consumption of beef by the Army, whose specifications are fairly high and quite rigid. Those ranchers who are so situated that they can "finish" all or part of their cattle mainly on local feeds supplemented with molasses and bagasse should develop such a practice and supply the good beef for the Honolulu market. The others will supply their local markets and the Honolulu market with ordinary range-fed beef, for which there will always be a large demand.

The chief reasons which seem to favor the development suggested are:

(1) The Territory as a whole profits by decreasing imports and increasing local production.

(2) By-products of the sugar industry, as molasses and bagasse, are utilized and their fertilizer value retained on the land. Under average cattle feeding conditions on the mainland, molasses fed at 15-20 per cent of the ration is worth as much pound for pound as corn or barley. If corn were 70 cents per bushel, molasses would then be worth \$20.00 per ton.

(3) Cattle from Island ranges are marketed at from two to five years of age, the average apparently being at about three years. Cattle may be finished in dry-lot and marketed at 2 to 2½ years or even less, with greater profits, other things being equal, from the quicker turn-over. This would allow the range to produce more animals on the same area.

(4) Many of the Island ranches have improved their breeding herds with pure-bred beef bulls to the point where steers of excellent beef type are being produced. It is a distinct loss to market such animals without a better realization of their hereditary possibilities in good or choice beef production.

(5) It seems possible, with an abundance of cheap feed, that Island beef could also be shipped to the Mainland, after the industry develops and after the local market is supplied.

#### ENERGY IN MOLASSES

The practicability of the development of this industry lies partly in the great amount of cheap energy which exists in the sugars left in waste molasses. These sugars are easily digested and utilized by the animal, and surplus salts and other materials not needed by the animal are excreted. Molasses can be fed in amounts as high as 30-40 per cent of the ration without any ill effect from the large amount of sugars and salts it contains. Its efficiency is a little less at these higher levels than at the 15-20 per cent levels.

Molasses contains plenty of energy in the form of carbohydrate, but contains almost no protein, a substance which is essential as building material for growth, and which is necessary in lesser amounts throughout the mature life of the animal. Molasses must therefore be supplemented with a high protein feed. Growing leafy forages, such as grasses, contain some protein of optimum quality, i.e., containing all the amino-acids; and range-fed animals grow satisfactorily on such feed, provided only that there is enough of it, and the water supply is sufficient. It is possible, indeed, to bring beef animals to a mature, fattened, well-finished condition on grass alone, but there are only a few areas in the world where soil, rainfall and other climatic conditions make pastures good enough, luxuriant enough in growth, and at the same time cheap enough to allow this result. It is not enough, therefore, for the ranges of Hawaii to improve the pastures by sowing better quality grasses. Such improvement of the ranges is much to be desired in order to nourish the breeding herd properly and allow the young stock to grow well and not too slowly.

The production of good beef, however, demands a finishing period during which the feed is properly balanced, abundant and palatable, no long walks are required to get it, and quiet, shade, and shelter, if necessary, promote the consumption of plenty of feed and the laying on of fat and mellowing of the tissues. Under these conditions cattle are content, become more tame, gain weight rapidly ( $2\frac{1}{2}$  to 3 pounds per day), and the skin and coat become mellow and glossy, things which delight the hand and eye of the experienced cattleman because they mean that the desired effects are going on under the skin. Range animals so fed not only gain rapidly during a 3- to 4-month period, but are worth considerably more per pound on the market.

#### DIGESTIBILITY OF BAGASSE

In order to make quite clear how by-products of the sugar industry fit into the picture, it should be stated that ruminants (as cattle and sheep) in which the first stomach or paunch is very large, obtain considerable energy from the bacterial fermentation of cellulose, pentosans and other plant materials which are not digestible by the digestive enzymes of the body. Organic acids and gases are produced by this fermentation, the former of which are oxidized in the body to yield energy. This fermentation is a normal process by which the fibrous tissues of plants are softened and cell walls broken down, thus allowing the digestive juices to get at the nutrients within the cells. Such materials as straw, corn stalks and cane bagasse, which yield some energy in the digestive tract of horses and similar animals, are more completely fermented in the paunch (rumen) of cattle and sheep. The exact amount of energy yielded in this process can not be known without careful calorimetric digestibility tests, but it is quite considerable, even though much of it is changed into useless gases. Thus bagasse is not only an excellent carrier for the molasses it will absorb, but it also contributes food value to the ration.

As stated above, both molasses and bagasse supply energy but almost no protein. A mature beef animal does not need as much protein as a growing calf, but must have some. The best protein supplement available in this Territory is soybean oil meal, which is imported at a price which is quite reasonable. It happens to be true that the proteins of the soybean are the best of all seed proteins known. Therefore a

smaller amount of this supplement is necessary than of other less efficient protein carriers, such as linseed oil meal. Other protein supplements are possible, such as cottonseed meal, sunflower seed meal, or even fish meal. The last has not been used much for cattle feeding, but its animal protein is most efficient of all in feeding.

The most logical plan for supplying the protein supplement is the cooperative development of the soybean industry in the Islands. Soybeans are used considerably and wisely for human food by Oriental peoples. The oil pressed from the seeds is readily marketable at 10 cents per pound, the same price as linseed oil. The residue after the oil is removed from soybeans is the very excellent high protein feed which we have been discussing. There are other protein crops which can be developed in various localities, such as pigeon peas, which are now being grown on some ranches. If such legume pastures grow luxuriantly on any ranch, the cattle may there secure their protein by grazing over a not too large paddock, with the bagasse and molasses fed in a trough or bunk. Otherwise, it is best to have the protein supplement mixed with the bagasse and molasses.

#### FEEDING EXPERIMENTS

Feeding experiments are now being carried on by several plantations and ranches in the Territory. A number of different rations are being compared for their relative efficiency in producing gains in the dry-lot feeding of range cattle. The University of Hawaii has carried on experiments with fattening cattle and dairy cattle, the rations including molasses and sometimes bagasse or pineapple bran. Further experiments are necessary in order to determine approximately the most efficient proportions of the feeds available. In localities where other feeds are available, the place in the scheme for their use should be determined.

The most logical "standard" by-product ration is molasses, bagasse and soybean oil meal, supplemented, if possible, with some grass or with cane tops. The latter are quite valuable as a supplementary feed, since, like all green leaves, they supply mineral matter, vitamins and high quality protein. The Honokaa practice of feeding work animals on this type of ration has demonstrated its feasibility. It has not shown us, however, what the most efficient proportion of the various ingredients may be.\* It is also true that the proportion of protein feed in this standard ration should be varied somewhat with the type, age, etc., of the animals being fed.

Probably the most satisfactory and economical method of obtaining this experimental information is by the cooperation of interested ranchers in different localities. There is plenty of market for beef from all who are able to develop this type of feeding. The various cooperators could be advised and their experiments correlated from a central point, as the Experiment Station, with the experiments so planned

\*A feeding experiment at the Station, using rabbits as a type of animal with a digestive system similar to that of the horse, has been under way for 9 months. The experimental ration contains 55 per cent ground bagasse, with molasses, soybean oil meal and cane tops. On this ration the animals do their daily two hours in the mechanical exerciser as satisfactorily as do the controls on a ration of alfalfa, barley and molasses.

that the ranches carrying on the tests will learn from them the most efficient method of getting the desired results under their own conditions and with their own local feeds. Each experiment will also contribute to the general knowledge of cattle feeding in the Islands and all will benefit therefrom. Such experiments with relatively few cattle (several groups of about 20 animals each) should precede any large-scale feeding operations.

During the experimental stage finished steers or carcasses should be sent to the Honolulu market in order to develop a knowledge of and demand for such beef. If the chain store markets and other good markets have demonstrated to them that Island beef can be produced exactly as good and as uniform in quality as imported carcasses, there is no reason why local producers should not have this market. There is also no doubt as to the ability of Island cattlemen to produce this quality of beef from their present herds.

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## The Third International Congress of Soil Science

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By FRANCIS E. HANCE

Delegates from sixty countries assembled at Oxford, England, on Monday, July 29, 1935. Stewards met the trains, conveying the guests first to reserved accommodations in the various residential Halls of the University and later to the reception room and headquarters of the Congress in the Hall of Wadham College. Delegates were registered, supplied with publications and literature bearing upon the Sessions of the Congress, given membership identification lapel buttons and assigned in groups to student guides, the latter to function for the period of the Congress.

The meetings opened on Tuesday, July 30, at Rhodes House, with an Address of Welcome by the Vice-Chancellor of Oxford University, followed by the Presidential Address of Sir E. J. Russell and reports by other officials of the Congress.

The work of the Congress was started on Wednesday, July 31. Deliberations of the Congress were held in plenary sessions at Rhodes House in the mornings, followed in the afternoons by separate or joint sessions of six commissions and three sub-commissions.

The work of the Society by Commissions was distributed by topics among the following subjects:

1. Soil physics
2. Soil chemistry
3. Soil microbiology
4. Soil fertility
5. Soil genesis, morphology and cartography
6. Application of soil science to land amelioration
  - 5a. (Sub-commission) Alkali soils
  - 5b. (Sub-commission) Forest soils
  - 6a. (Sub-commission) Peat soils.

Participation by a single individual in all of the work of the commissions and sub-commissions was, of course, a physical impossibility because six or more commission groups worked simultaneously.

Early on the morning of each session day, a detailed program for that day was posted at headquarters. Delegates were privileged, therefore, to arrange their attendance at sessions best suited to their needs and interest.

Several outstanding figures in the world of soil science attended the Congress. Personal discussions were made possible at informal gatherings of members during the late afternoon teas after commission sessions had closed.

Deliberations of the Congress were concluded on August 7, 1935. The meetings at Oxford were followed by a post-congress tour of Great Britain in which studies were made of the soils of North Shropshire, Wales, The Highlands, North Eastern Region, Central Valley and Southern Uplands of Scotland; Yorkshire and East Anglia. Following a luncheon at the University Farm, the excursion terminated at Cambridge on August 23, 1935.

Many papers were presented to the Congress on a great variety of subjects. Of these, a large number was of particular interest and value to plantation men. Rather than present an abstract of the contributions, it is the author's belief that perhaps a more concise digest of the meetings attended may be found in the notes recorded at the end of each session day. This plan of presentation allows introduction of the highlights of discussion which took place among small groups at the many informal occasions where impromptu meetings were held. The "notes," supplemented by quotations, are rearranged by topics.

#### *The Soil Survey:*

The importance of soil-water relationships was emphasized for an initial survey of agricultural land. Attention was given to presence of salt, alkali or other soluble mineral deposits and the physical or chemical effects of these materials upon the colloidal structure of the soil under natural or artificial irrigation. The profile examination and chemical analysis of the soil contributed not only to the water-relationship study but furnished a means of soil classification and definition according to types and in addition gave valuable information as to the suitability of the land for the crops designed to be grown upon it.

Field experiment was looked upon as an essential part of an agricultural survey. It was proposed that field experiment might serve as a complement to chemical analysis. A few urged that field experiment should replace chemical analysis. One prominent member stated that in his experience field experiment had been found severely limited in its serviceable applications. He proposed that the profile and chemical survey be employed as the basis for laying out and placing the field experiment and interpreting the resultant data.

Mitscherlich tests, aspergillus studies and other methods of soil analysis received favorable mention in this connection. In an informal discussion it was proposed that after the determination of soil profile characteristics, soil-water relationships and total chemical analyses of new land, the agriculturist could employ field experiments, Mitscherlich tests and reliable rapid chemical soil and crop analyses in correlation, with crop yields, in deciding upon the program of fertilization for any given soil type, district and crop. It was agreed that an understanding and full cooperation between the water scientist, the agriculturist and the chemist were essential to the success of intensive agricultural pursuit.

#### *Soil Reaction (pH):*

The agricultural significance of soil reaction was shown to be important and to have a direct relation to physical and chemical soil characteristics. pH considerations, base replacement, phosphate fixation, movement of soil nutrients and textural differences in soil appear, to a great extent, to be interdependent.

The rather general and present trend toward the extensive employment of acid-forming nitrogen fertilizers, particularly on normally acid soils, were shown by replicated field experiment at Rothamsted to have brought about acute infertility. The oxidation in the soil of the ammoniacal fertilizers to acids appears to result in the replacement of soil bases such as potassium, calcium, magnesium, etc., by the hydrogen of these acids so that the bases are present in solution and are readily lost

by leaching. When reactions such as these occur in the field and have progressed for a number of years, the fact may be determined by a study of the soil reaction.

Under the caption of "Calcium" it will be shown that a restoration of fertility may be accomplished by rigidly controlled liming of naturally acid fields that have been rendered infertile by ammoniacal dressings. A reclamation of this character is always accompanied by a partial and, on occasion, by an almost complete neutralization of the soil acidity induced by the ammoniacal fertilizer. Control in this case employs pH determinations. Other important physical and chemical considerations are involved as a result of the lime treatment. These will be discussed later. To offset the accumulative development of acidity in soils as a result of employing acid-forming nitrogenous fertilizers, the English fertilizer manufacturers have produced a number of new ammoniacal fertilizer mixtures which contain either neutralizing or non-neutralizing compounds of calcium. These new fertilizers are designed for use on fields which have received a primary corrective lime treatment. It has been claimed that when used as a substitute for sulfate of ammonia, one of these new products, for instance, "nitrate of chalk," will *not* contribute to an increase in soil acidity, the loss of nutrient bases nor to a reversion to infertility. It should be emphasized that the cumulative acid effect of the ammoniacal fertilizers in the field became acute at Rothamsted after a continuous and yearly repetition of the fertilizer applications for a period greater than fifty years; also that the soils receiving this treatment were originally acid in reaction and became considerably more acid in the interim. The severity of the phenomenon in localities other than England and Continental Europe was questioned by a few Congress members. The fact remains, however, that no data were submitted by those dissenting, as far as the author is aware, which had been obtained from a similar, continuous fertilizer plot study having a duration of fifty years or more.

The crop (hay) in the Rothamsted experiment, fertilized by sulfate of ammonia, was admittedly one sensitive to increases in soil acidity. Some crops, particularly sugar cane, exhibit a high degree of tolerance to acid soils. It may be found, therefore, that the acid tolerance of the crop, in addition to the pH and the chemical composition of the soil, govern to some extent the period involved in the appearance of soil infertility when induced by the uncompensated use of acid-forming nitrogenous fertilizers.

Relative to corrective amendments used to overcome soil acidity, one investigator reported satisfactory results with carbonate of lime when mono-ammonium phosphate had been used to fertilize a soil under 5.5 pH. Another found a mixture of superphosphate and sulfate of ammonia equally effective for cereal crops. In both cases increases in soil acidity had apparently been checked. Nitrate of Chalk, mentioned previously, is manufactured on a large scale in Great Britain. It consists of a mixture of ammonium nitrate and calcium carbonate. This product is marketed in the form of small granules and is surprisingly stable under conditions of ordinary exposure. It has been reported, however, that Nitrate of Chalk gives off a small amount of ammonia on long storage. A similar product manufactured in Norway consists of equal parts of calcium and ammonium nitrates. This mixture also is furnished in globular pellets and is said to be quite satisfactory to handle even in damp weather. pH determinations by B. A. Keen of the soils of the "classical"

experimental plots at Rothamsted and Woburn gave results of interest. A study was made of the changes produced in the soil by the long and continuous use of various fertilizers. In regard to the liming of soils it was shown that changes in the pH values which occurred in the field were less than those determined by lime requirement tests in the laboratory because of subsoil acidity. It was shown that changes in reaction on the surface layers of soil due to liming penetrated to and affected somewhat the lower horizons to a depth of three feet. Also that these subsoil changes in pH were contributory to differences in soil texture which were not apparently simple functions of pH. Quoting from the report:

The top soil, although the most acid, showed no flocculation, probably owing to the protective action of the humus colloids; the  $4\frac{1}{2}$ - to 9-inch depths showed distinct flocculation but the suspension remained turbid; the lower depths flocculated completely and immediately, possibly owing to the accumulation of calcium and aluminum ions leached down from the very acid surface layer. These textural differences will affect air and water movement within the soil; they are important, indirect factors in the relationship between soil reaction and crop growth.

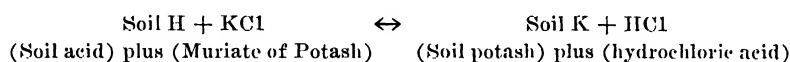
### *Calcium:*

Base exchange soil phenomena have been studied by many investigators and have been discussed in the literature for years. The useful and practical applications of base exchange principles in soils studies have been increasing rapidly with the development of this branch of soil science. The mechanism of exchange is a simple one in principle but in the soil of the field, exchange reactions are governed by several factors, among which one of the most important is the base calcium.

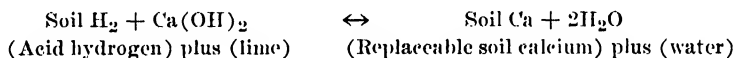
Calcium is an essential plant nutrient. Some of its compounds have wide application in correcting excessive soil acidity. Ample exchangeable calcium in the soil exercises a direct control upon the establishment of potash and other bases in the nutrient reserve; it acts as a buffer in retarding the development of soil acidity; it contributes to a desirable condition of soil tilth and granulation and it establishes a "seat of exchange" where applied potash fertilizer may be held secure from wasteful leaching and yet function normally as a freely available source of the nutrient. One very prominent member, an outstanding authority on base exchange, decried the common practice of wholesale liming of fields without any degree of control. He suggested that calcium be looked upon as a plant nutrient as well as a soil amendment and that it should be employed much the same as other nutrients, except where its compounds were used to neutralize soil acidity and to establish a "seat of favorable exchange." Discussion of the subject brought out observations upon the beneficial effects of lime when applied to soils as a corrective after years of leaching by heavy rainfall. It was stated that the exchangeable bases in clay soils may be regarded as bound to distinctive alumina-silicate clay acids. During leaching of this soil by rainfall in humid climates exchangeable bases are carried away and replaced by acid soil hydrogen. Lime applications assist in the amelioration of soil colloids and insure enrichment of the clay-humus soil fraction with subsequently added potash. It was shown that neutral potash fertilizers (muriate of potash is an example), when applied to acid soils deficient in calcium, do not "form a supply" in the soil and will not do so in some soils until the excessive acidity is reduced by adequate treatment with lime. This may be considered a questionable claim, perhaps, for it could be assumed that since the greater amount of acid hydrogen in the

soil is essentially exchangeable hydrogen, it should be "replaced" by applications of other bases, potash compounds, for instance, since these are used in ordinary fertilization. Such is not the case, however. An illustration presented by D. J. Hissink at the Congress makes this point quite clear. He stated:

Manuring with potassium is almost always done with neutral potassium salts soluble in water, chiefly muriate and sulfate of potash. Experiments have shown that in this way no supply of potassium is formed in the soil in strongly acid soils. It is not until the soil reaction of these strongly acid soils has been made less acid by means of liming that a stock of potassium is formed in the soil with potassium fertilization. This phenomenon may be explained in the following manner. In slightly acid to slightly alkaline humus-clay soils, the potassium of the potassium fertilizer finds enough lime in the soil for exchange. In strongly acid soils, however, the potassium of the potassium fertilizer has to exchange itself against the (acid) hydrogen of the soil and the soil hydrogen can only be replaced to a slight extent by treatment with solutions of neutral salts. The following reaction is what takes place:



Theoretically speaking, it is easy to understand that, owing to the strength of the hydrochloric acid formed, this equilibrium shifts only slightly to the right. If, however, the test is carried out in such a way that a solution of muriate of potash is poured over the soil, so that the hydrochloric acid formed is leached out, there is no apparent reason why this process should not continue. Yet this is not the case. Experiments were made by Ramann in which he leached out the soil with a few hundred liters of muriate of potash solution, without succeeding in replacing more than a very slight amount of (acid) hydrogen with potassium. . . . There is only one (practical) way to bring about an immediate and complete replacement of the acid soil hydrogen, and that is the treatment of the soil with basic compounds or hydroxides (calcium hydroxide or mill lime, raw rock phosphate, coral rock or coral sand, etc.). In this case the soil hydrogen combines with the hydroxide (the coral or the phosphate) to form harmless water, e.g.



Dr. Hissink concluded his remarks in this manner:

When the soil, this living organism, which is able to render plant growth possible, has lost the adsorbing soil complex, it becomes a completely sterile thing, a dead body, which is of no further value to plant life. It is, therefore, one of the most important tasks of agriculture to prevent the disintegration and ultimately the complete destruction of the adsorbing soil complex. Although perhaps other measures, such as, for instance, rational plowing, may be appropriate, I will only point out in this address that an ample application of lime to the adsorbing soil complex is the measure par excellence, for the reason that the adsorbed calcium does most to check the destructive activity of water (water containing carbon dioxide or water flowed upon treatments of acid-forming fertilizer salts). In other words, adsorbed calcium gives the greatest stability to the adsorbing soil complex. And so I may best conclude this address by repeating in a slightly altered form Tacke's pronouncement with which I began a treatise on the soil lime question twenty years ago. (*The lime question is by far the most difficult and at the same time by far the most important problem of the theory of manuring.*)

Summarized, it may be restated that in the regions of heavy rainfall in humid climates, soil acidity tends to become more acute by the leaching downward of exchangeable bases, and the substitution in their place of acid hydrogen. Without the stabilizing influence in the soil of the exchangeable bases, chiefly calcium, losses occur, by leaching, of applied potash fertilizer and other nutrient constituents. To insure adequate adsorption of potash fertilizer by a crop in such a soil it becomes

necessary to add to that soil sufficient lime to partially neutralize the increased acidity and to establish a seat of exchange where the potash and other bases may be held against leaching and in a form usable by the crop. The long continued application of acid-forming nitrogenous fertilizers on any soil, other than coral lands and alkali districts, may be expected in time to result (1) in the development of increased soil acidity, (2) in failure to hold potash against leaching, and (3) in loss of exchangeable bases. The remedy appears to consist in the use of lime in one or more of its several combinations simultaneously with, or in addition to, the acid-forming fertilizers. Lime applications to the soil have also been found beneficial when previous action of the elements and time have increased soil acidity and have resulted in the removal from the soil of the greater part of its exchangeable basic fraction. Unquestionable support of these conclusions has been found in experimental field studies in England and in Holland.

On the naturally acid soil plots at Rothamsted which had become infertile and more acid as a result of fifty years of continuous fertilization with sulfate of ammonia, in a complete fertilizer mixture, exchangeable calcium had disappeared almost entirely from the soil and the loss of potash by leaching had become severe. Reclamation was made by application of lime. Chemical studies conducted in this research gave added weight to the premise that the alkaline lime treatment had replaced the excess of acid soil-hydrogen, thus allowing the base, calcium, to become a useful part of the replaceable soil complex. Further evidence was found to substantiate the belief that subsequently applied potash then found sufficient calcium for exchange and the reconstructed soil complex was accordingly supplied with the required plant potash. It is assumed, of course, that other basic nutrients are similarly involved—all to the apparent advantage of soil and crop.

An interesting study was concluded recently at Rothamsted in which 55 years of continuously cultivated wheat and barley on plots at Woburn gave additional information of value relevant to acid nitrogenous fertilization, lime treatment of soil and soil organic matter. During fifty years of this period without applications of farmyard manure, one-third of the soil organic fraction was lost. It required seven tons of farmyard manure per year under the same crop, on neighboring plots, to maintain the soil organic fraction at a fixed concentration. Superphosphate did not influence soil reaction nor affect the base status of the soil in others of these plots during fifty annual applications. A number of plots which had received sulfate of ammonia for fifty years and had become acutely infertile were subsequently divided for attempted reclamation by lime. Study of these plots by E. M. Crowther yielded data which showed that the rate of loss of lime became less as the soil developed increased acidity. This finding illustrates the correlation between diminishing calcium reserves and increased soil acidity. In a study of the comparative effect of nitrate of soda and sulfate of ammonia on the lime reserves of these soils, the following conclusion was reached. If lime were to be used to maintain the chemical composition of soil and crop at concentrations comparable to those of soil and crop under nitrate of soda fertilization, the amount of lime which would be required would be equivalent to the combined nitric and sulfuric acids produced in the oxidation of the sulfate of ammonia used. Unfortunately this concise statement is rendered somewhat obscure by the additional finding that nitrate of soda has

a much more beneficial effect on plants growing in very acid soils than can be accounted for simply by the reduction in loss of calcium by leaching.

An American investigator, M. F. Morgan, presented data compiled from a lysimeter test conducted on soils kept free of vegetation. A study had been made of the comparative effects upon uncropped soil of sulfate of ammonia, urea and nitrate of soda. The conclusions reported agreed in many respects with the findings of European investigators. In the study with sulfate of ammonia it was reported that:

1. In spite of applications of potash to the soil, exchangeable potash was depleted.
2. A reduced degree of base saturation occurred.
3. Aluminum and manganese salts appeared in drainage water.
4. The loss of calcium in the leachate was large.

Where urea was used the results obtained were similar in effect to those observed with sulfate of ammonia. The effect upon soil bases, however, was less severe.

With nitrate of soda, it was reported that:

1. Exchangeable sodium increased slightly.
2. A minimum loss of other bases occurred.
3. Applied potash was held in the exchangeable form.
4. Less calcium was leached from the soil than in other cases.
5. There was no marked change in total base exchange capacity.

The advantageous role of calcium in soil-base exchange reactions has been shown to be an important one. The organic fraction of the soil also appears to have an important bearing upon base exchange matters. One delegate to the Congress, Prof. Sante Mattson, an outstanding authority on soil chemistry, presented a paper upon pH and base saturation of the podzol profile. He stated:

Some of the soil bases exist in combination with organic acids of the more simple and soluble type. But, while considerable quantities of bases may be stored up in the humus, these bases or metal cations are of little direct benefit, because their release involves a still further lowering of the pH. If, however, the conditions which favor the production of humic and other organic acids should be succeeded by conditions which favor the complete decomposition of the organic matter into water and carbon dioxide, we can easily foresee some of the immediate results. After the destruction of some of the humic acids the residue would attain a higher degree of base saturation and the pH would increase. The acid hydrolysis would then cease, less bases would be lost by leaching, and the soil complex would become positively saturated with bases throughout the profile.

The practice of incorporating filter cake, molasses and cane trash with the soil is not an uncommon one in Hawaii. It was shown in Prof. Sante Mattson's contribution that the return of vegetable residues to the soil occasions an increase in the supply of bases and tends to counteract the development of soil acidity.



*"Slowly" Available Soil Phosphate:*

We have been accustomed to differentiating soil phosphate as "difficultly soluble" and "readily soluble (or available)." That there must exist in the soil an intermediate supply of phosphate between these two extremes of solubility, there can be but little doubt. It was of interest, therefore, to learn that E. Truog and L. A. Dean, having determined the occurrence of this intermediate supply, had submitted to the Congress the findings of their research. They described a unique quantitative method of ascertaining simultaneously the concentration in the soil of "readily available" and "slowly available" phosphate. Their method may be found in the Transactions of the Congress. A brief description follows: A high ratio of weakly acid solvent to soil is employed. Hence, while tricalcium phosphate is readily dissolved, allowance is made for measurement of rate of hydrolysis of phosphate from basic iron phosphate. The solvent does not dissolve sufficient iron from the soil to interfere with colorimetric readings. The phosphate thus dissolved is considered as readily available. If, however, the amount found is low, they show the advantage of ascertaining whether the phosphate found came largely from a "moderate" supply of tricalcium phosphate or a "good" supply of basic iron phosphate. Basic iron phosphate would be more desirable in such a case, they stated, because of its slower rate of solution during the period of crop growth. To learn the nature of the phosphate supply, the analyses are twice repeated exactly as in the first case with the exception that the soil sample is reduced in amount by one-half and again by one-fourth of the original amount employed. If it is found that a marked falling off in concentration of phosphate occurs in the subsequent analyses, then tricalcium phosphate predominates in the sample. If, on the other hand, the concentrations found do not become markedly less, the results indicate that basic iron phosphate predominates.

*A Note on Manganese:*

The laboratory of Miss Brenchley at Rothamsted has been the scene of researches of world-wide significance upon the role of the minor elements. J. P. Martin, W. T. McGeorge, L. E. Davis, R. K. Conant, W. W. G. Moir and others have shown in Hawaii that manganese is essential in the metabolism of sugar cane and that it functions specifically in the control of Pahala blight of sugar cane. It was of interest, therefore, to learn in Miss Brenchley's laboratory that a great many other crops also require manganese in minute amounts and for the same apparent reasons that it is required by sugar cane. A new feature of the requirement, however, that the writer believes has escaped our attention, was the finding that the actual amount of manganese present in the soil was not critical but that the proportion of this element present in exchangeable form was the factor which governed its useful function. When naturally present, or when once established in the soil, it appears that replaceable manganese continues to exercise its desirable effect only as long as its concentration is maintained at some, as yet undetermined, but exceedingly low value.

*The Estimation of Active Chemical Factors in Plant Nutrition:*

Members of the Congress were given opportunity to become acquainted with

Dr. M. F. Morgan's Rapid Chemical Tests for determining the availability in the soil of the major plant nutrients. The Morgan analyses differ from ours in methods of soil extraction, elaboration of detail in analytical procedure and in specialization of determination for the type of information sought.

The Morgan determinations are described as "micro-chemical tests"—ours as "rapid chemical analyses."

Morgan employs a "universal" solvent for all "tests." It is a half normal solution of acetic acid buffered at 4.8 pH with sodium acetate. This single soil-extracting solution is employed for the development of colorimetric or turbidity tests in determining nitrates, nitrites, ammonia, phosphorus, calcium, magnesium, aluminum, manganese, iron and sulfates. A single extraction of a level teaspoonful (5 grams) of soil and 10 ml of universal extracting solution is said to remove from the soil approximately three-fourths of the nitrates, one-half of the ammonia, calcium, potassium, magnesium and manganese, one-tenth of the aluminum and one-twentieth of the phosphorus. Charts are provided for estimating the test results "in pounds of nutrient per acre." Ten drops of the solution extracted from the soil specimen are used in all tests except that for nitrates, where one drop is sufficient. For ammonia, four drops only are required and for aluminum two drops have been found to be ample.

The nitrate test employs the diphenylamine reaction, while nitrites are estimated by means of Lombard's reagent, hydrochloric acid and sodium hydroxide. Ammonia is determined by the well-known Nessler's reagent. Phosphorus is estimated by the ceruleo-molybdate reaction. Potassium is identified as potassium cobaltinitrite, the precipitation being accomplished by employing isopropyl alcohol. Calcium is estimated as oxalate while a color development is used for magnesium, either paranitrobenzene-azo-resorcinol or Titan yellow reagent with subsequent addition of sodium hydroxide. Hematein is used in the aluminum test and the soil extract is made weakly alkaline for the manganese test with benzidine. Sulfates are precipitated with barium chloride, ferric iron is shown by color changes upon the addition of potassium sulpho-cyanate and ferrous iron by potassium ferri-cyanide.

In the majority of cases but one drop of an appropriate reagent is required to develop a readable test, the final reading being obtained within one or two minutes. An experienced operator of the Morgan "tests" may cover the entire series of estimations on as many as seventy-two soils in one working day—a total of about 864 nutrient determinations per day.

In support of the Hawaiian plantation analysts who are performing not "micro-chemical tests" but duplicated and checked quantitative rapid chemical analyses of soils, plant materials, mill by-products and irrigation waters, the following statement, taken from a record available at the moment, may be made: "During the harvesting season one analyst has collected and composited representative soil samples on two-acre blocks from 2600 acres, air-dried and screened the specimens and has analyzed each (in duplicate), determining potash, calcium, phosphate, phosphate fixation, soil reaction and soil nitrogen."

## SUMMARY

This paper is essentially a resumé of notes made by the author while in attendance at the Sessions of the Third International Congress of Soil Science in England. Topics discussed are: Soil Survey, Soil Reaction, Acid-Forming Nitrogenous Fertilizer, the Functions of Calcium in (1) Base Replacement, (2) Correction of Soil Acidity, (3) Amelioration of Soil Colloids, and (4) In the Conservation of Applied Potash; Manganese and Estimations of Active Chemical Factors in Plant Nutrition.

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## Notes on *Pythium* Root Rot

### VIII

#### ABSORPTION OF ESSENTIAL ELEMENTS BY SEGREGATED ROOTS OF SUGAR CANE

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By C. W. CARPENTER

The nutrition of the sugar cane plant and its effect on the susceptibility of the roots to *Pythium* root disease was recently discussed by the writer (1). It was mentioned in that article that small shoots of sugar cane, excised from germinated cuttings, had been grown with one or more roots detoured to suitable containers in order to supply specific chemical elements. These experiments, wherein the plants were grown to large size either in soil or in culture solutions, with individual roots supplying elements deficient in the medium in which the remainder of the root system was growing, contributed to our knowledge of the probable effect of localized fertilization in the field.

Hance (3) has devised fertilizer briquettes designed to supply nutritive elements deficient in the soil and to meet problems of fixation of certain nutrients. Such elements applied to the soil in the usual way might be fixed and remain relatively unavailable to the plant. The behavior of the plants in the experiments with individual roots detoured as mentioned above supported the view of Hance that localized fertilization in the cane furrow was feasible, provided the soil moisture was not a limiting factor.

In studies already referred to (1) a series of cane plants was grown in phosphate-deficient soil with one root of each plant detoured into a solution of sodium phosphate. The improved growth of the plants thus supplied with phosphorus in contrast to control plants with no external increment of phosphorus indicated that the one root with its abundantly developed branches supplied this element in sufficient amount to materially benefit the plants. When one root was functioning in this manner there appeared to be a decrease in the severity of the *Pythium* root rot\* in the remainder of the root system in the soil.

Similar results were observed with cane plants grown in culture solutions deficient in either phosphorus or potassium, and with individual roots detoured respectively into solutions containing these elements. The growth of such cane plants during a period of six months was comparable to that of control plants in complete nutrient solution, and to plants of similar age in the field. In contrast, plants grown in solutions deficient in either phosphorus or potassium were less vigorous, and the root systems were conspicuously affected with *Pythium* root rot. The "firing" of the leaves and the red mottled midribs characteristic of potassium deficiency of sugar cane as reported by Martin (5) and Hartt (4) were apparent in the plants growing in the solution lacking potassium, but no symptoms of potassium deficiency

\* Caused by *Pythium graminicolum* Subr.

were observed in the plants where one root was permitted to develop in distilled water containing potassium.

The above experiments indicated that different roots could supply the plant with specific elements available in their vicinity and that soil solutions corresponding to a complete nutrient solution might not be required at any one point in the soil for the plant to function normally. These observations led to the experiments described in this paper, wherein each of the nine elements, considered essential for normal cane growth, was supplied to individual plants through a separate root. No record of the growth of plants in this manner, which may be referred to as segregated root nutrition, has come to the attention of the writer.

The complete nutrient solution used was modified from the formula described by Martin (5). The table shows the composition of the solution and the parts per million of each element. Solutions of the chemically pure compounds numbered one to nine in distilled water were used for the separate roots. The compound used in each unit solution supplied one of the nine nutrient elements under consideration.

Solution No.	Source of Essential Element	Element	Mol. Conc.	P. P. M.
1	Na NO <sub>3</sub> (Sodium nitrate)	N	.009	126
2	NaH <sub>2</sub> PO <sub>4</sub> . H <sub>2</sub> O (Mono-basic sodium phosphate)	P	.001	31
3	K Cl (Potassium chloride)	K	.004	156
4	Ca Cl <sub>2</sub> . 2 H <sub>2</sub> O (Calcium chloride)	Ca	.006	240
5	MgSO <sub>4</sub> . 7 H <sub>2</sub> O (Magnesium sulphate)	Mg	.001	24
6	Fe SO <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> . 6 H <sub>2</sub> O (Ferrous ammonium sulphate)	Fe	...	10
7	Mn SO <sub>4</sub> (Manganous sulphate)	Mn	...	0.25
8	H <sub>3</sub> BO <sub>3</sub> (Boric acid)	B	.00002	0.216
9	Na <sub>2</sub> Si O <sub>3</sub> . 9 H <sub>2</sub> O (Sodium silicate)	Si	.00001	0.28

Cuttings of the cane variety H 109 were allowed to germinate in wet bagasse. Shoots measuring about 12 to 16 inches from base to leaf tips were severed from the cuttings, rooted in complete nutrient solution and permitted to develop therein. The solutions were renewed once a week. After six weeks, when the plants had become well established in the complete nutrient solution, three plants of uniform size were selected. One was retained as a control plant and grown continuously in complete nutrient solution in the usual manner. All but ten roots of each of the other two plants were excised. Nine of the roots of each plant were detoured respectively into the nine solutions shown in the table, with one reserve root of each plant detoured into distilled water.

The plants were set for anchorage in dry quartz sand in aluminum pots provided

with suitable outlets. The quartz sand was previously washed with hydrochloric acid and then with distilled water. The photographs show the arrangement of the roots, glass tubing and bottles of nutrient solutions. The assembly was enclosed in a suitable box to protect the roots from the light and prevent the growth of algae. The bottles of brown glass were provided with rubber stoppers fitted with two



Fig. 1. Cane plant grown from a small severed shoot with nine roots segregated respectively in solutions of nine essential elements.

glass tubes; one tube was curved with the short arm inserted through the rubber stopper; the other, a straight tube, permitted the use of a smaller glass tube in siphoning and renewing the nutrient solution at weekly intervals. When the bottles and vertical tubes were filled to the proper level, the solution partly filled the curved tubes so that the tips of the selected roots could be immersed in the solution. The roots grew and followed the bore of the tubes into the bottles. A complete solution was maintained in the bottles for two weeks thus allowing the roots to grow through the tubes and become established in the solution in the bottles. Thereafter the appropriate solution containing but one essential element was used in each bottle.

Normal and comparable growth of the three plants prevailed for the first four months. Thereafter the two plants with all roots in the closed bottles fell slightly

behind in comparative vigor of growth; at six months they were noticeably smaller than the control plant. All, however, had developed stalks about one inch in diameter and five feet in length (exclusive of the leaves). The slower growth of

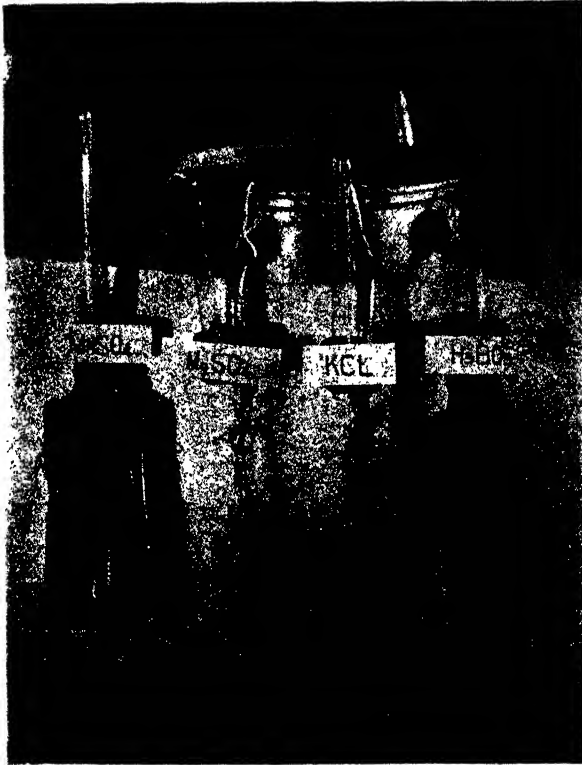


Fig. 2. Root systems developed from individual roots segregated respectively in solutions supplying manganese, magnesium, potassium, and boron.

the plants with segregated roots might be attributed to the restricted root development and the consequent reduced absorption area in the bottles; to the very limited capacity of the nine roots to transmit water and nutrients into the stalk base; and to the lack of aeration of the culture solutions, as compared to the exposed surface of the culture solution in the control jar.

The photographs show the root systems which developed in the several solutions of the essential elements. The dissimilarity of the roots in the various solutions was striking. The experiment has been repeated with similar results. The largest mass of white, vigorous roots, completely filling the contour of the bottle with a thick mat (Fig. 3) developed in the calcium chloride solution in duplicate sets of the two experiments. These root masses were in marked contrast to those which developed in any of the other solutions (Figs. 2, 3 and 4). It is not assumed that these odd experiments are conclusive or that the observations are sufficiently complete for

far-reaching interpretations. Factors other than the presence of the one element, essential to plant growth, in each of the nine solutions, e. g., concentrations and pH values, may have influenced the sizes and types of the root masses. No doubt a



Fig. 3. Root systems in solutions supplying respectively, iron, calcium, and nitrogen. (See Fig. 2.)

portion of the growth of the plants may be attributed to the nutrients stored by the plants during the early stages of the experiments.

It is thought that experiments of this nature may provide useful information regarding the cane plant. Cooke (2) reported increased growth of sugar cane and Sudan grass following large applications of phosphate to certain soils where *Pythium* root rot was a factor in growth depression. The experiments detailed herein led to other tests\* which supplemented the observations of Cooke and showed that part of the response accompanying large applications of phosphate was due to the calcium portion of the fertilizer. In pot tests with the Hamakua growth-failure soil, applications of phosphorus and calcium tend to increase the resistance of cane and Sudan grass to *Pythium* root rot.

\* Discussed in the Director's Monthly Report of this Station for July and December, 1935.





Fig. 4. Root systems in solutions supplying respectively, nitrogen, phosphorus, and silicon. (See Figs. 2 and 3.)

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## Cane Growth Studies

### THE DOMINATING EFFECT OF CLIMATE

By R. J. BORDEN

It is extremely doubtful if many of our own agricultural workers have fully recognized the dominating effect that even such small differences in climate as exist throughout the sugar cane districts in Hawaii can have upon the results of their efforts to produce sugar in the field. U. K. Das has repeatedly called attention to various weather relationships that exist, and in 1934 he reported\* the results of a pot experiment which prompted us to plan another and more elaborate test of a similar nature. This new test† was designed to note the effects of a difference in the climatic environment upon the growth and production of sugar cane, using different soils, different varieties, and when different amounts of plant food were made available.

#### THE PLAN AND CONDITIONS

*The Climatic Conditions:* Since facilities were available for this study at both Makiki and the Manoa Substation, where the elevation is 40 and 550 feet respectively, and since these two locations enjoy differences in their daily and annual amounts of rainfall, wind, temperature and sunlight, the various treatments that are listed hereafter were duplicated at each of these places in order to provide a series of comparisons that would allow us to interpret the effect of some of these climatic influences.

In order to narrow down the sum total effect of all climatic factors, a protected area was chosen at each location where wind would not be likely to be a mechanical-effect factor. To offset any possible deficiency of soil moisture, all treatments were irrigated periodically and sufficiently to keep the plants growing; hence a lack of rainfall was not a growth-effect factor. We are chiefly concerned, therefore, with the factors of temperature and sunlight. Previous records of the sunlight differences show that Manoa receives approximately 40 per cent less sunlight than Makiki, being more generally overcast, especially in the afternoons. A comparison of the average rainfall and temperature for the two locations would show that Manoa has an annual rainfall of over 200 inches as compared with less than 50 inches at Makiki, and that the average minimum and maximum temperatures at Manoa are in the neighborhood of 67° and 79° respectively, as compared with 68° and 85° at Makiki. During the particular 14-month period through which the canes in this study were growing, the following conditions were recorded:

\* "A pot experiment with cane grown in the same soil but under different climatic conditions" by U. K. Das, *The Hawaiian Planters' Record*, Vol. XXXIX, pp. 26-29.

† H.S.P.A. Experiment Station Project A-105, No. 43.

Location	Total Rain- fall	No. of days with rainfall exceeding					Mean Min. Temp.	Mean Max. Temp.	Total Day- Degrees
		4"	3"	2"	1"	½"			
Makiki	46.96	1	0	1	5	13	67.5°	84.6°	6445
Manoa	198.94	5	7	7	34	46	67.4°	80.2°	4488

**Soils:** Two distinctly different soil types were selected: (1) The Manoa soil is a yellowish-brown silty loam, with a granular structure which makes it porous and well-drained. It is a soil that has been formed, in place, probably from coarse, volcanic ash under conditions of rather heavy rainfall. Thus it is deficient in available basic constituents, especially potash and calcium. It has a very high capacity to fix phosphate. The availability of its phosphoric acid is considered to be rather low, and it has responded to applications of phosphate fertilizers. It has a fair amount of available nitrogen and is quite high in organic matter. In the field it has a pH of about 5.4. It has supported cane growth very feebly, even when well fertilized, and hence it has been considered a "poor" soil for sugar cane. (2) The Makiki soil is a chocolate-brown loam of a much finer structure, which takes up water and drains more slowly when wetted, and packs quite firmly, but without cracking, as it dries. It is an alluvial soil which has a large supply of available phosphoric acid, a medium-high supply of available potash and calcium, but a very small amount of available nitrogen and a low content of organic matter. It has a pH of about 7.2 in the field. This Makiki soil is considered a very "good" cane soil and produces very heavy crops of cane.

Mitcherlich tests of the two soils used in this study gave the following results and indications of their fertility status:

Plant Food	Manoa Soil (pH 5.4)		Makiki Soil (pH 7.2)	
	(lbs. per Acre-Foot)	Status	(lbs. per Acre-Foot)	Status
Nitrogen .....	217	High	28	Low
Phosphoric acid .....	62	Doubtful	494	Very high
Potash .....	464	Doubtful	835	High

After removing the surface foot of soil from each field and thoroughly mixing them, each well-mixed soil was then put into large concrete tubs (2' x 2' x 2'). It was firmly tamped in the bottom half of each tub in order to prevent excessive leaching and to be more nearly like the soil conditions of the upper two feet of field soil. To the Manoa soil only, a heavy application of raw rock phosphate was made just under the seed before planting. This was done in order to insure an adequate supply of phosphoric acid to the cane which would be growing in this extremely high phosphate-fixing soil.

**Varieties:** Three cane varieties were chosen: (1) H 109 was selected because of its excellent record when grown on Makiki soil at Makiki and the general knowledge that it has performed well under climatic conditions similar to those at Makiki but has not done well in Manoa and similar climes. (2) Striped Tip was selected because it has performed fairly satisfactorily under conditions like those at Manoa. (3) POJ 2878 was chosen because it was one of the varieties used by Das in his preliminary study, and also because it was thought to be a variety that would perform quite satisfactorily under both Makiki and Manoa conditions.

*Planting:* In order to insure a full and comparable stand, ten single-eye cuttings of the variety desired were planted in each tub. These were later thinned so as to leave the best six plants for the basic stand. Thereafter all shoots were allowed to grow.

*Fertilization:* Because the number of containers available was limited, the differential fertilization was given to only one of the varieties that was grown. Hence we supplied the variety POJ 2878 with two levels of fertilization: an amount of nitrogen, phosphoric acid and potash from ammonium sulphate, superphosphate, and muriate of potash respectively, which we knew to be wholly inadequate for optimum growth was supplied every second month to one series of POJ 2878 plants, while an amount of the same materials which we deemed more than fully ample for maximum growth was given every month to a second series of POJ 2878. This same "ample" fertilization was supplied to all series planted with the H 109 and the Striped Tip canes. Actually, the canes that were "amply" fertilized received four times the amount of plant food which was given to those that were "inadequately" fertilized.

All fertilizer applications were made on the surface of the soil and an irrigation was given to put the material immediately into solution. The first application was not made until the cane was two month old since the cane growth was very slow at the start in October and November. Thereafter, the monthly applications were continued until one month before the cane was harvested.

#### DISCUSSION OF THE RESULTS

At the age of 14 months the canes were cut off and topped, the millable cane weighed and crushed, and the crusher juice analyzed for the various constituents which we desired to study. The results have been summarized in Tables I to V inclusive which follow. All weights and analyses given are the average of three pots of each treatment. Standard errors are included for the guidance of those who may wish to use them.

Table I gives the average pounds of millable cane harvested from all varieties, soils, and fertilizer differentials when grown at Makiki as compared with the duplicates grown at Manoa. The cane yield differences quite definitely favor the Makiki climate and the average relative yield of cane shows a crop at Makiki that has produced 2.41 times the yield at Manoa. Thus it would appear that 100-ton cane at Makiki, which can easily be produced, would be compatible with 41.5-ton cane at Manoa, which is probably seldom exceeded there. In other words, it would seem that regardless of or in spite of the soil, the variety, or the fertilization which may be given to sugar cane, a climatic handicap can dominate cane yields to such an extent that even the best agriculturist will do well to obtain much more than 40 per cent of a possible Makiki tonnage under the restricted sunlight and lower maximum temperatures that exist in cane-growing regions which are similar to Manoa. In Fig. 1, we offer an excellent example of the relationship that existed between the weekly growth measurements of the variety H 109 on two distinct soil types, and the effective temperature (as measured by day-degrees) at the two locations during the period when these measurements were being made.

When given ample fertilizer, H 109 produced the most, and Striped Tip the least cane under the favorable climatic conditions at Makiki. Both H 109 and Striped Tip were equally able to make use of the poorer conditions at Manoa and they both were apparently slightly better than POJ 2878 there.

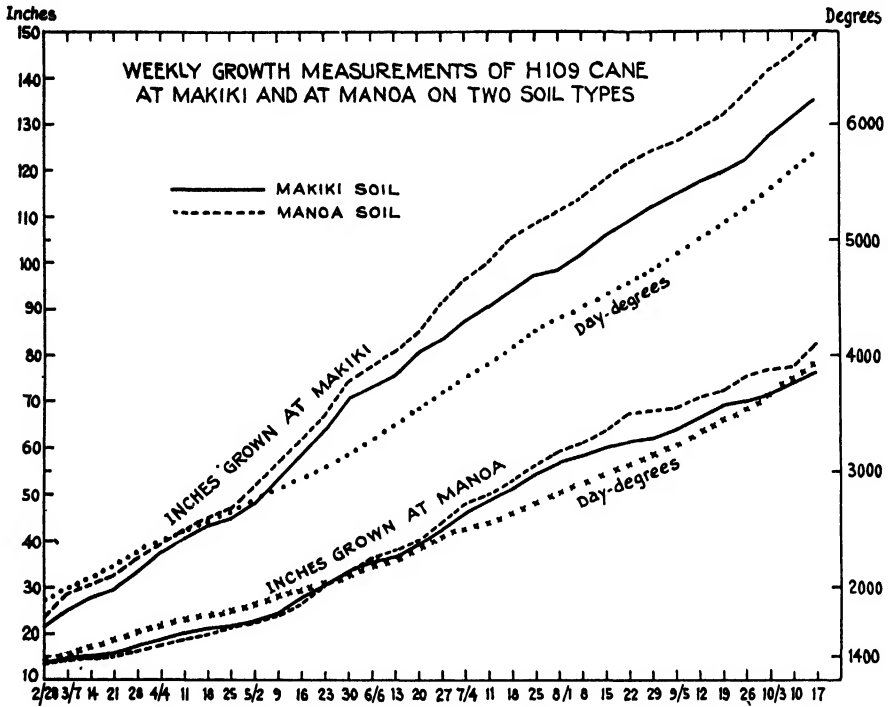


Fig. 1.

We have summarized in Table II the average "yield per cent cane" or pounds of sugar per 100 pounds of cane, in order to show that there was some 29 per cent more sugar per unit of cane produced at Makiki than at Manoa. This evidence of a better quality of cane at Makiki in seven out of the eight comparative combinations of variety, soil, and fertilization, would tend to show that perhaps climate dominates these other factors in determining cane quality as well as in affecting cane yields. It is particularly interesting to note the good quality of 14-month-old cane harvested at Makiki in December, when it is remembered that heavy nitrogen fertilizer applications were made monthly, right up to the time of harvest, and that there was no artificial ripening-off.

Disregarding for the present any difference in soil, both of the amply fertilized H 109 and POJ 2878 canes grown at Makiki had a very similar sugar content, of 12.24 and 11.99 per cent respectively, and both were better than Striped Tip with its 10.75 per cent average. On the other hand, the Striped Tip cane grown at Manoa had a slightly better sugar content than H 109, 9.0 per cent against 8.8, while the correspondingly heavily fertilized POJ 2878 with 8.0 per cent sugar was the least efficient storer of sugar at that location.

TABLE I  
THE CANE YIELDS

Variety	Soil	Fert.	Average lbs. millable cane:		Difference favoring Makiki climate:	
			grown at Makiki	grown at Manoa	in lbs. of cane	Rel. Diff.
H 109	Maki.	Am.	81.8±3.9	35.4±1.6	46.4±4.3	2.31
"	Man.	"	105.0±3.4	31.5±.8	73.5±3.5	3.33
St. Tip	Maki.	"	44.9±4.2	32.0±1.9	12.9±4.6	1.40
"	Man.	"	71.4±2.8	34.2±2.7	37.2±3.9	2.09
POJ 2878	Maki.	"	69.9±7.3	20.6±5.8	49.3±9.3	3.39
"	Man.	"	87.0±1.0	28.6±3.2	58.4±3.3	3.04
POJ 2878	Maki.	In.	25.4±1.8	17.5±1.6	7.9±2.4	1.45
"	Man.	"	38.2±3.8	16.8±.9	21.4±3.9	2.28
						Average 2.41

Abbreviations used: Var. = variety                      Fert. = fertilization  
Maki. = Makiki                      Man. = Manoa  
Am. = amply fertilized  
In. = inadequately fertilized  
Rel. Diff. = Relative difference or  $\frac{\text{Makiki cane yield}}{\text{Manoa cane yield}}$

Note: Averages are presented with their S.E. (Standard Error).

TABLE II  
YIELD PER CENT CANE

Variety	Soil	Fert.	Average yield % cane:		Equivalent Q.R.'s:		Difference favoring Makiki climate:	
			at Makiki	at Manoa	at Makiki	at Manoa	in yield % cane	Rel. Diff.
H 109	Maki.	Am.	12.99±.42	9.53±.46	7.7	10.5	3.46±.62	1.36
"	Man.	"	11.50±1.02	8.07±.80	8.7	12.4	3.43±1.30	1.42
St. Tip	Maki.	"	9.91±.47	10.40±.89	10.1	9.6	-0.49±1.0	.96
"	Man.	"	11.69±.78	7.62±.81	8.5	13.1	4.07±1.13	1.53
POJ 2878	Maki.	"	12.26±.52	8.39±1.38	8.2	11.9	3.87±1.47	1.46
"	Man.	"	11.72±.70	7.60±1.14	8.5	13.2	4.12±1.34	1.54
POJ 2878	Maki.	In.	12.30±.68	12.11±.68	8.1	8.3	.19±.96	1.01
"	Man.	"	11.66±.92	10.91±.25	8.6	9.2	.75±.95	1.07
								Average 1.29

Equivalent Q.R. = "quality ratio" which is the reciprocal of "Yield per cent cane."

Sugar yields are largely influenced by a combination of both cane growth and its sugar content. Under the more favorable Makiki climate, we have produced more than three times the amount of sugar than the less favorable Manoa climate has given us (see Table III). This is the combined result from more cane and a better cane quality at Makiki. Variety comparisons quite clearly indicate that H 109 was able to take best advantage of the favorable conditions, while Striped

Tip was best adapted to deliver sugar from the more restricted amounts of sunlight and temperature received at Manoa. Thus we note further specific and measured evidence of a variety adaptation to climatic differences.

TABLE III  
THE SUGAR YIELDS

Variety	Soil	Fert.	Average lbs. sugar:		Difference favoring Makiki climate:	
			produced at Makiki	produced at Manoa	in lbs. sugar	Rel. Diff.
H 109	Maki.	Am.	10.63±.68	3.37±.20	7.26±.71	3.16
"	Man.	"	12.14±1.79	2.54±.21	9.60±1.91	4.78
St. Tip	Maki.	"	4.47±.61	3.46±.58	1.01±.84	1.29
"	Man.	"	8.38±.86	2.93±.45	5.45±.97	2.86
POJ 2878	Maki.	"	8.64±1.20	1.87±.68	6.77±1.38	4.62
"	Man.	"	9.34±.71	2.19±.07	7.15±.71	4.27
POJ 2878	Maki.	In.	3.15±.38	2.14±.30	1.01±.48	1.47
"	Man.	"	4.53±.77	1.94±.02	2.59±.77	2.33
Average						3.10

The influence of climate upon the phosphate and potash concentration in the cane plant, as indicated by the percentages of these mineral elements found in the crusher juice is seen in Table IV. Relatively 19 per cent more phosphate and 50 per cent more potash were found per unit of juice in the canes grown at Manoa, and where adequate fertilization was given there was only one instance out of twelve comparisons where the percentage of these minerals in the juice was less at Manoa than at Makiki.

TABLE IV  
PHOSPHATE AND POTASH IN THE CRUSHER JUICE

Variety	Soil	Fert.	Average % $P_2O_5$ in juice:		Difference favoring Manoa climate:		Average % K <sub>2</sub> O in juice:		Difference favoring Manoa climate:	
			at Makiki	at Manoa	in % $P_2O_5$ in juice	Rel. Diff.	at Makiki	at Manoa	in % K <sub>2</sub> O in juice	Rel. Diff.
H 109	Makiki	Am.	.078	.085	.007±.012	1.09	.18	.22	.04±.02	1.22
"	Manoa	"	.014	.021	.007±.003	1.50	.05	.15	.10±.02	3.00
St. Tip	Makiki	"	.128	.134	.006±.008	1.05	.26	.27	.01±.02	1.04
"	Manoa	"	.046	.061	.015±.005	1.33	.08	.12	.04±.02	1.50
POJ 2878	Makiki	"	.082	.099	.017±0.18	1.21	.31	.27	-.04±.03	.89
"	Manoa	"	.024	.026	.002±.007	1.08	.09	.21	.12±.02	2.33
POJ 2878	Makiki	In.	.137	.123	-.014±.004	.90	.30	.28	-.02±.02	.90
"	Manoa	"	.025	.034	.009±.006	1.36	.08	.09	.01±.01	1.12
						Average	1.19		Average	1.50



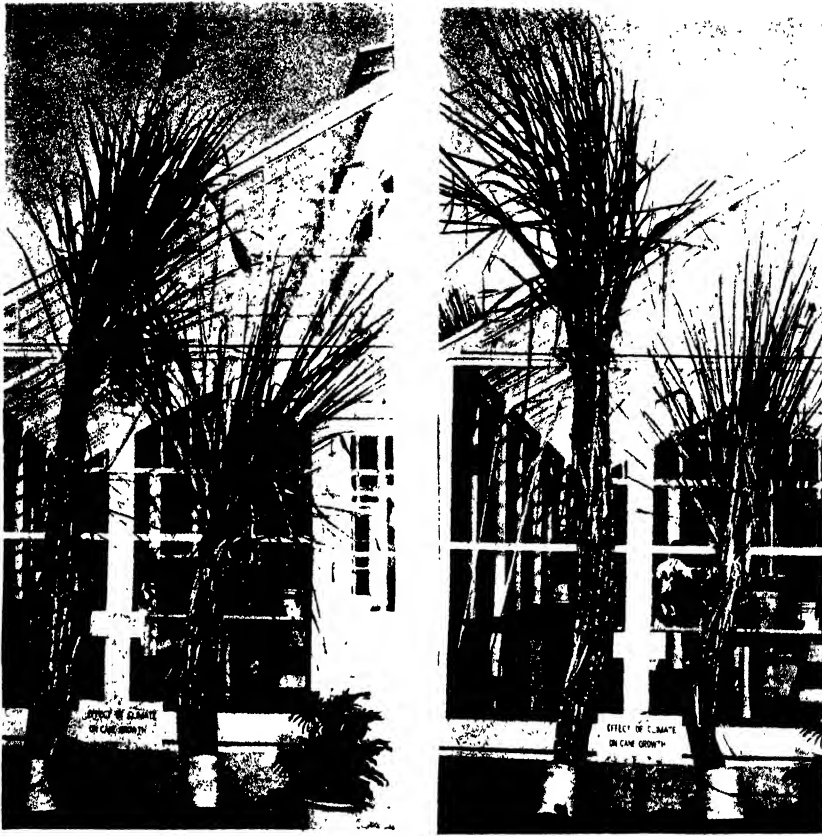


Fig. 2. H 109 cane grown with ample fertilization: (at the left) on "good" Makiki soil at Makiki and at Manoa respectively; (at the right) on "poor" Manoa soil at Makiki and Manoa respectively.

TABLE V  
CHANGES IN THE SOIL pH

Variety	Soil	Fert.	Original pH	pH of soil at harvest	
				Cropped at Makiki	Cropped at Manoa
H 109	Makiki	Am.	7.2	6.3	6.1
St. Tip	"	"	"	6.2	6.1
POJ 2878	"	"	"	6.1	5.8
POJ 2878	"	In.	"	7.0	6.6
H 109	Manoa	Am.	5.4	5.5	5.1
St. Tip	"	"	"	5.3	4.8
POJ 2878	"	"	"	5.2	4.7
POJ 2878	"	In.	"	6.5	5.5
			Average	6.3	6.0
					5.6

Table V shows the changes in pH of the soils that occurred while these crops were growing on them. The series grown at Manoa has become more generally acid than the Makiki series. Where the wholly adequate amounts of fertilizer were supplied, we note the following changes: (a) the original pH of 7.2 of the Makiki soil has dropped to 6.2 at Makiki and to 6.0 at Manoa; (b) the original pH of 5.4 of the Manoa soil has changed to 5.3 at Makiki and to 4.9 at Manoa. It is quite

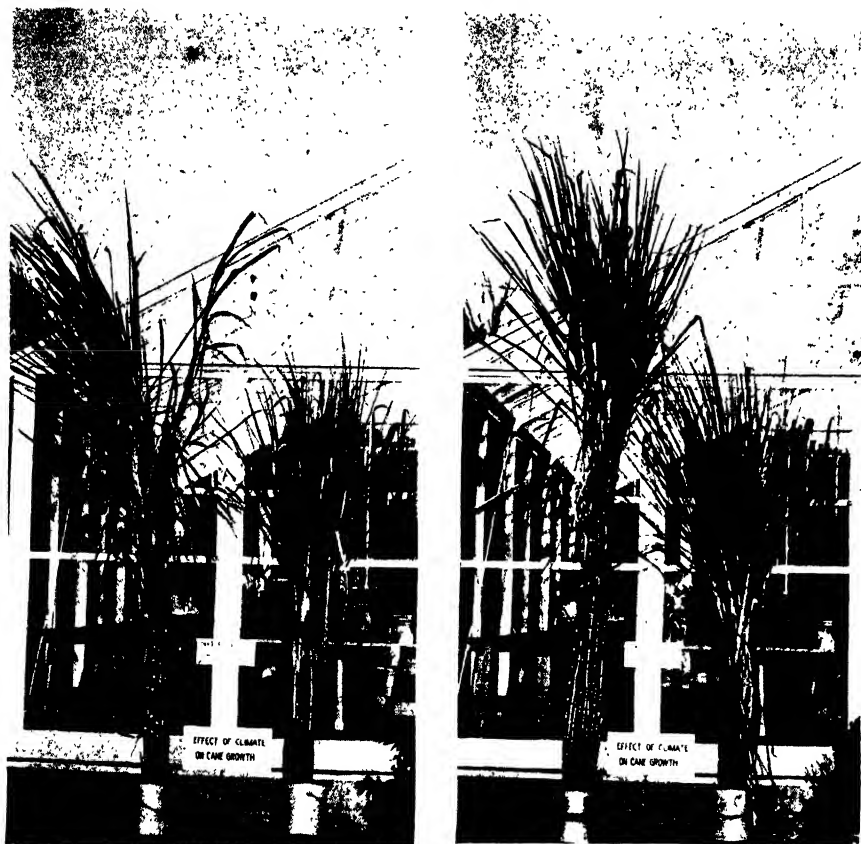


Fig. 3. Striped Tip cane grown with ample fertilization: (at the left) on "good" Makiki soil at Makiki and at Manoa respectively; (at the right) on "poor" Manoa soil at Makiki and Manoa respectively.

likely that the increase in the acidity of these soils has been due to the large monthly applications of ammonium sulphate that were made. At Makiki the difference between a pH of 7.2 and 6.2 in the Makiki soil could be equivalent to an increase of only about 5 active acidity units,\* while the difference between the pH of 5.4 and 5.3 for the Manoa soil cropped at Makiki is equivalent to an increase of 10 active acidity units. When these same comparisons are made for the series grown at

\* E. T. Wherry (Bull. No. 4, Am. Hort. Society) uses directly related numbers termed "acidity units" which indicate the "active acidity" or the amount of hydrogen ions in the solution that are free to exert the effects commonly classified as acidity. They help us to more clearly visualize what the differences in pH may represent.

Manoa, the increased acidities for the Makiki and Manoa soils were of the nature of 9 and 85 active acidity units respectively, i.e., the acidity of the Manoa soil was increased under the Manoa climate to more than nine times its increased acidity under the Makiki climate.

#### OTHER RELATIONSHIPS

In addition to a study of the effects of climate, the data offer an opportunity to note several other interesting relationships.

1. *Soils*: The two soils used in this study were, as has been indicated, quite different both in their physical and original chemical composition. Both soils were fertilized with what was considered to be an abundant supply of plant food (except in one series of treatments) and this was supplied each month the crop was growing. Hence there should have been no real plant food deficiency in either soil.

The results (see Table VI) show that the relative cane yields from the Manoa soil averaged 24 per cent more than from the Makiki soil. However, it will be seen that the Manoa soil at Makiki produced 40 per cent more cane, while on the same soil at Manoa the increase was only 8 per cent over the Makiki soil. Was the better physical condition of the Manoa soil responsible for these gains? Can this better physical condition of a soil be a factor of more importance where superior climatic conditions are available for the production of larger cane crops? Conversely, there would appear to be slightly more sugar per unit of cane (yield per cent cane) for the crops grown on the Makiki soil. Although the individual comparisons do not show yield differences that are large enough to be significant, the fact that seven of these eight comparisons show a higher relative "yield per cent cane" figure in favor of the Makiki soil, would indicate a possible influence of soil on cane quality. We do not know why this should be so. Was this poorer quality from the Manoa soil caused by the heavy application of rock phosphate (with its large calcium content) that we mixed into the surface foot of the Manoa soil in our attempt to insure an adequate supply of phosphate for the cane in this high phosphate-fixing soil; might this poorer quality have been associated with a possibly higher content of calcium and nitrogen in the plant? Was the better quality from the Makiki soil in any way related to the larger percentages of both phosphate and potash that were found in the crusher juices of all the treatments grown on this soil? (Our usual experience has been that the quality is more apt to be inversely related to the percentage of phosphate and potash in the crusher juice.)

The individual differences in the sugar yields produced from comparisons made on these two soils are perhaps not significant amounts, although it may be significant that we find the Manoa soil netted considerably more sugar when the cane was grown thereon under the favorable climatic conditions at Makiki.

2. *Varieties*: In addition to what has already been said about the varieties studied in this test, the comparisons as arranged in Table VII are of interest.

H 109 was superior to both POJ 2878 and Striped Tip at both Makiki and Manoa, and yet it was apparently a more economic user of both phosphate and potash. POJ 2878 was better than Striped Tip when grown at Makiki but the reverse was true when these two varieties were compared at Manoa. The POJ 2878 took up less phosphate but more potash than the Striped Tip.

TABLE VI  
EFFECTS SECURED FROM DIFFERENT SOILS

Variety	Grown at	Fert.	Difference favoring Manoa soil:		Difference favoring Makiki soil:		Difference favoring Manoa soil:		Difference favoring Makiki soil:	
			in lbs. cane	Rel. Diff.	in yield % cane	Rel. Diff.	in lbs. sugar	Rel. Diff.	in P <sub>2</sub> O <sub>5</sub> in juice	in K <sub>2</sub> O in juice
H 109	Makiki	Am.	23.2±5.2	1.28	1.49±1.10	1.13	1.51±1.91	1.14	.064	.13
"	Manoa	"	— 3.9±1.8	.89	1.46±.92	1.18	— .83±.29	.76	.064	.07
St. Tip	Makiki	"	26.5±5.1	1.59	—1.78±.91	.85	3.91±1.06	1.88	.082	.16
"	Manoa	"	2.2±3.3	1.07	2.78±1.20	1.37	— .53±.73	.85	.073	.15
POJ 2878	Makiki	"	17.1±7.4	1.24	0.54±.87	1.14	.70±1.39	1.08	.058	.22
"	Manoa	"	8.0±6.6	1.39	.79±1.79	1.10	.32±.68	1.17	.073	.06
POJ 2878	Makiki	In.	12.8±4.2	1.50	.64±1.14	1.06	1.38±.86	1.44	.112	.22
"	Manoa	"	— 0.7±1.8	.96	1.20±.72	1.11	— .20±.30	.91	.089	.19
Averages			10.6	1.24	.90	1.12	.78	1.15	.077	.15
Significance			Odds 60:1		Odds 20:1		Odds 9:1			
			Average Rel. Diff. (favoring Manoa soil)		Average Rel. Diff. (favoring Makiki soil)		Average Rel. Diff. (favoring Manoa soil)			
			Grown at Makiki 1.40		Grown at Makiki 1.05		Grown at Makiki 1.38			
			Grown at Manoa 1.08		Grown at Manoa 1.19		Grown at Manoa .92			

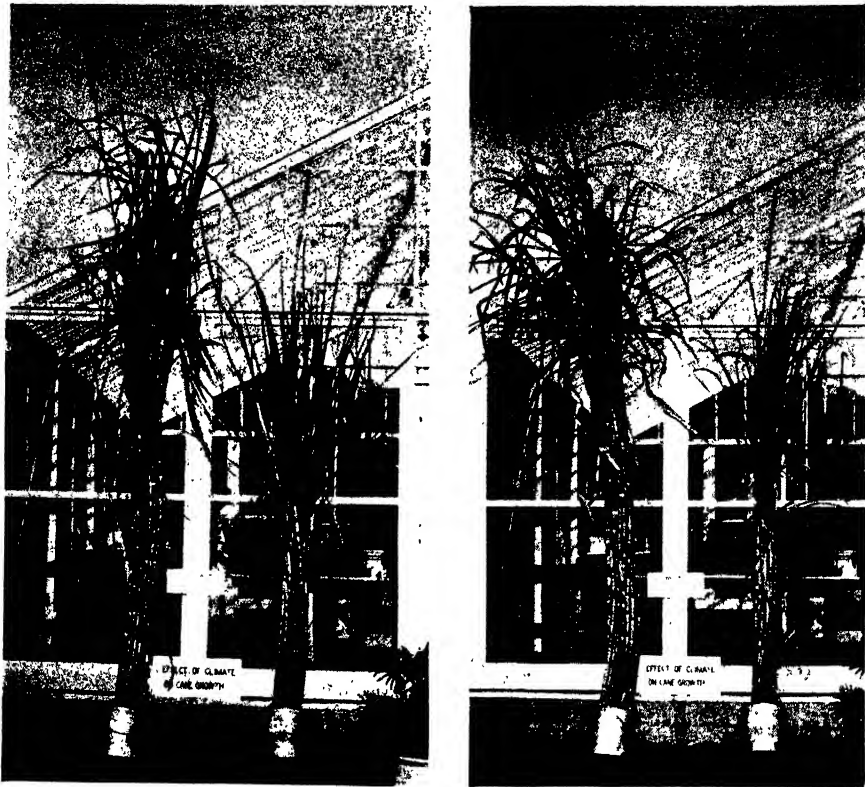


Fig. 4. POJ 2878 cane grown with ample fertilization: (at the left) on "good" Makiki soil at Makiki and at Manoa respectively; (at the right) on "poor" Manoa soil at Makiki and Manoa respectively.

TABLE VII  
RELATIVE DATA CONCERNING VARIETIES

	Grown at	Soil	Cane	Yield %		$P_2O_5$ in Juice	$K_2O$ in Juice
				Cane	Sugar		
a. Relative yield	Makiki	Makiki	1.17	1.06	1.23	.95	.58
	Makiki	Manoa	1.21	1.08	1.30	.58	.56
	Manoa	Makiki	1.72	1.14	1.80	.86	.82
	POJ 2878	Manoa	1.10	1.06	1.22	.81	.71
	Average		1.30	1.08	1.39	.80	.67
b. Relative yield	Makiki	Makiki	1.82	1.31	2.38	.61	.69
	Makiki	Manoa	1.22	.99	1.45	.30	.63
	Manoa	Makiki	1.12	.92	.98	.63	.82
	St. Tip	Manoa	.84	1.06	.87	.34	1.25
	Average		1.25	1.07	1.42	.47	.85
c. Relative yield	Makiki	Makiki	1.56	1.24	1.93	.64	1.19
	Makiki	Manoa	1.22	1.00	1.11	.52	1.12
	POJ 2878	Manoa	.64	.81	.54	.74	1.00
	St. Tip	Manoa	.84	1.00	.75	.43	1.75
	Average		1.07	1.01	1.08	.58	1.27

TABLE VIII

## RELATIVE EFFECT OF INCREASED FERTILIZATION ON POJ 2878

	Grown at	Soil	On Cane	On Yield % Cane	On Sugar	On P <sub>2</sub> O <sub>5</sub> in Juice	On K <sub>2</sub> O in Juice
Relative yield	Makiki	Makiki	2.75	1.00	2.74	.60	1.03
	Makiki	Manoa	2.28	1.00	2.06	.96	1.12
Ample fertilization	Manoa	Makiki	1.18	.69	.87	.80	.97
Inadequate fertilization	Manoa	Manoa	1.70	.70	1.13	.77	2.33
	Average		1.98	.85	1.70	.78	1.36

3. *Fertilization:* A glance at Table VIII will show that when POJ 2878 was given an ample supply of fertilizer on either soil type used, a gain in yield of cane and sugar over the inadequately fertilized series was quite evident. Such gain, however, was much greater when the cane was grown at Makiki, and hence we have further evidence that the limiting growth factor at Manoa was quite likely climatological.

It is of special interest to note that the heavier fertilization did not affect the

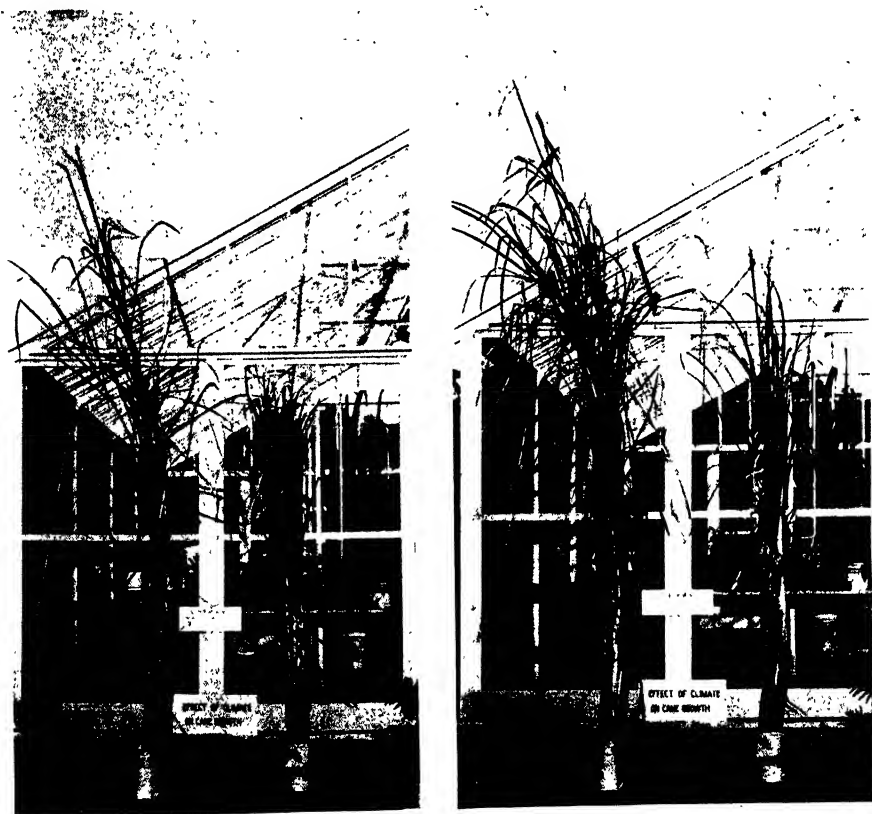


Fig. 5. POJ 2878 cane grown with inadequate fertilization: (at the left) on 'good' Makiki soil at Makiki and at Manoa respectively; (at the right) on 'poor' Manoa soil at Makiki and Manoa respectively.

quality of the POJ 2878 cane that was grown at Makiki. On the other hand, the quality of the cane grown at Manoa was adversely affected by the large fertilizer applications. Thus it may be argued that under those conditions where sunlight is limited and temperatures are low, too much fertilizer may quite likely result in a poor cane quality, but where these climatic factors are not apt to be limiting growth factors, the same degree of concern about poor juices resulting from heavy fertilization need not be necessarily felt.

#### CONCLUSION

This study of the effect of climate upon cane and sugar yields has given us further evidence of the dominating influence that climatic factors can have upon our field results. When we disregard the soil, variety, and fertilization differences, it would appear that we may expect at Manoa, where temperatures are lower and skies quite apt to be overcast throughout a large part of the growing period, only about one-third as much sugar as from the Makiki area that is more favorably situated. However, this effect is apparently subject to some improvement, especially when advantage may be taken of the ability of some cane variety to utilize the limited light and heat conditions more efficiently.

It would also appear that perhaps some soil condition, probably physical, might be modified in such a way that a greater efficiency would be secured from different climatic environments. The closer grained Makiki soil produced 8 per cent more sugar at Manoa than the more granular, open type of Manoa soil; conversely, this loose, granular Manoa soil was 38 per cent better than the more compact Makiki soil, when it was cropped at Makiki.

Certainly we will have to recognize that cane which is being grown under unfavorable climatic influences cannot be given the excess of available plant food, without suffering a poorer quality, that it is possible to give to cane that is growing where there is little or no limiting growth-effect due to climate.

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# Rat Control Investigations at The Lihue Plantation Company, Ltd.

By THOMAS G. ECKART

## INTRODUCTION

Many steps have been taken during the past few years to improve the poison bait methods of rat control throughout the Territory. A great deal of outstanding work has been done by Peniberton, Barnum and Doty of the Experiment Station, H. S. P. A., and their pioneering in poisoned grain baits is the backbone of the recent investigations at The Lihue Plantation Company, Limited. A great deal of credit must be given these men for the foundation they laid.

Everyone realizes that rats are carriers of disease, and that rats do a certain amount of damage. How many of us have stopped to figure out rat damage in dollars and cents, and how many of us realize what a negligible expense proper rat control is compared to the savings resulting from that control?

The purpose of this paper is not only to point out the value of rat control but also, to briefly summarize the recent poison bait studies\* conducted at The Lihue Plantation Company, Limited, and the changes in control policy resulting from these studies.

## THE VALUE OF A DEAD RAT

According to the joint statements of the United States Chamber of Commerce and the Bureau of Biological Survey, rats and mice do approximately five hundred million dollars damage annually in the United States, each rat doing damage to the extent of at least two dollars. "Tests of captive rats show that every rat will consume sixty pounds of grain or other foodstuffs in the course of a year" (1).

It is a well-known fact that rats in sugar cane fields destroy a great deal more cane than they actually eat. Rat-eaten cane stalks sour and decay and if the damage is at all severe the result is a decreased cane tonnage and poor juices. To growers of sugar cane a dead rat is worth several times more than two dollars, but in arriving at "The Value of a Dead Rat" there is another factor to be considered besides the actual damage done today. Rats breed four or five times a year and each litter consists of from 6 to 10 young, divided equally as to sexes. A pair of rats may thus produce a large progeny in a year (2). Theoretically then, a dead rat is worth a great deal more than two dollars.

## RATS AT LIHUE

The common rat of Lihue fields is the gray rat, *Rattus norvegicus* (Erxleben), reported as being the most difficult species to control.

During the 1935 harvesting season of The Lihue Plantation Company, Lim-

\* Detailed counts have been omitted from this paper. Only final summaries are presented.



ited, 90,664 rats were caught in the harvest fields of the Lihue and Hanamaulu divisions and 10,248 were caught elsewhere on the plantation—a total of 100,912 rats. These rats were caught by two men who, with a shovel and a pack of dogs each, followed the loading machines through the harvest fields of the two divisions named. These men were paid by the day and by the rat tail. The 100,912 rats represented an investment of not over \$1,500.00 and a saving of \$201,824.00 at two dollars a rat. If these rats each did damage only to the extent of two cents, the money spent was still very worth while.

A total of 330,256 rats have been caught in the harvest fields of the Lihue and Hanamaulu divisions during the past seven years. A total of 115.5 *tons* of rats—worth at least \$660,512.00 to the plantation and representing an investment of not over \$8,000.00.

In the harvest fields of the Lihue division, where rats are very numerous because of the many stone walls, an average of 31.2 rats per acre of harvested cane was caught in 1935. The Hanamaulu harvest fields, during the same period, yielded an average of 10.3 rats per acre. Some rats are killed in “burning off”—a great many escape to adjacent fields and near-by stone walls and rock piles—so the average number of rats caught is a mere fraction of the population per acre. The difference in the above per acre averages is due, not only to the stone walls and rock piles of the Lihue division, but also to the difference in efficiency of the two rat men and their dogs and differences in poison bait control.

A record of the rats caught per day provides a means of studying rat population, and permits giving special poison bait attention to badly infested areas during the progress of the following crop. Besides, the rats caught in specific fields year after year provide a fair index of poison bait control efficiency.

### OBJECTIVES

The poison bait studies recently completed at The Lihue Plantation Company, Limited, were planned and conducted with the following objectives:

- (1) To arrive at an “effective take” or “kill” index, in terms of amounts consumed, for grain baits treated with various amounts of thallium sulphate.
- (2) To study various poison carriers and vegetable oils in the hope of finding a bait of outstanding rat acceptance.
- (3) To study the amounts of improved bait eaten per unit of time with the plan of adjusting the thallium sulphate concentration to secure a maximum kill.

### EFFECTIVE TAKE

Thallium-sulphate treated wheat (one pound of thallium sulphate to 1000 pounds of wheat), prepared by the Pacific Guano and Fertilizer Company, made up into waterproof (paraffin dipped) torpedoes has been used on this plantation for several years. A great many distributed torpedoes always remained untouched or slightly nibbled and we began to wonder just how effective our poison bait

control was. Recent studies by Garlough\* show 30 mg/kg to be a lethal thallium sulphate dose for rats disregarding possible food effects. A lethal dose for a large rat then, weighing 350 grams, under laboratory conditions,† is 10.48 mg. of thallium sulphate. At a ratio of one pound of thallium sulphate to 1000 pounds of wheat an average Lihue torpedo, weighing 9 grams, contained 9 mg. of thallium sulphate—only enough to kill small and medium-sized rats and mice providing they ate the entire torpedo or a very large portion of it. Very few torpedoes were eaten entirely—less than 10 per cent consumed to the extent of 50 per cent or more. The old, wheat torpedoes were not only too weak, they were not particularly attractive as a food.

The thallium sulphate concentration of whole wheat was doubled during 1935. Theoretically, a half of a whole wheat torpedo of this new strength should kill small and medium sized rats. A cage test in which 12 rats of various sizes were kept in separate cells, showed that half of one of these more powerful torpedoes would kill the small and medium sized rats but not the largest.

Early in 1936, when Lihue changed from whole wheat to rolled barley, the thallium sulphate concentration was again increased (one pound of thallium sulphate to 333 pounds of barley). Rolled barley is approximately 33 per cent lighter than whole wheat per unit volume and besides—rats do not eat the hulls. The average Lihue rolled barley torpedo weighs 6½ grams and contains 3.09 mg. of thallium sulphate per gram. Half of one of these barley torpedoes, then, is theoretically sufficient to kill any rat. Repeated cage tests using the new barley of increased thallium sulphate strength showed half a torpedo sufficient to kill rats of all sizes.

W. P. Alexander, Assistant Manager of Grove Farm Company, Limited, reports that missing torpedoes are generally entirely consumed. This he discovered by tying long strings to torpedoes and carefully retrieving the remains (generally paper shells) of those which were missing.

Throughout this investigation the 1 to 500 and 1 to 333 thallium sulphate concentrations were used. Reference to effective take means torpedoes which have been consumed to the extent of 50 per cent or more—or those which are missing. For the weaker of the two concentrations (one pound of thallium sulphate to 500 pounds of grain) the effective take is not absolutely true, as 50 per cent of a torpedo was not sufficient to kill all rats, but may be considered true for the experiments here discussed without appreciably affecting the results.

#### CORN OIL

Doty (3) (4) found an increase in take, at the Kailua Substation of the Experiment Station, H. S. P. A., by dipping torpedoes in corn oil. Experiments to study the effect of corn oil on wheat torpedoes were planned and set out. Our results corresponded closely to those obtained by Doty.

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\* F. E. Garlough—now conducting rat investigations throughout the Territory.

† A rat diet under field conditions may have some effect on the killing power of thallium sulphate.

## FIELD 5W HM.

	Effective Take*—Per Cent			
	Test No. 1—		Test No. 2—	
	2 days	4 days	2 days	4 days
Corn-oil dipped .....	60.5	71.4	71.2	75.4
Corn-oil sprayed .....	26.3	42.9	58.5	63.1
Untreated† .....	10.5	26.2	47.7	50.8

\* Effective take varies with rat population and therefore can *only* be compared within an experiment.

† The effective take of untreated wheat in these tests is extremely high due to an unusually large rat population in the area.

Tests conducted in other areas gave the same conclusive results. Corn-oil dipping was then made a standard plantation practice and other bait studies were commenced.

## GRAIN PREFERENCE

Reports of other investigators in the Territory and elsewhere to the effect that rats preferred rolled barley to wheat led to grain preference studies. Twelve healthy field rats (6 large and 6 medium sized) were placed in separate cages. Glass jars containing the following materials were placed in each cell.

- 1—Water
- 2—Barley (rolled)
- 3—Sunflower seed
- 4—Corn (cracked)
- 5—Wheat (whole)
- 6—Soybeans

The containers were not refilled. As the rats consumed the contents of one jar they were obliged to make a second choice, etc. In each cage all the rolled barley was thoroughly consumed first—only hulls remained. Second choice was a draw between sunflower seed and cracked corn. Third choice was wheat and the rats became very hungry before they touched it. The soybeans remained untouched and because the purpose of the test was fulfilled the rats were destroyed. This test was repeated with identical results; however, before changing our plantation practice from poisoned wheat to poisoned barley, further studies, under field conditions, were desired.

## CORN OIL INSIDE AND OUT VS. CORN OIL OUTSIDE ONLY

It seemed logical that if rats liked corn oil and they do, that they would eat more of a torpedo if the grain were saturated with corn oil. Doty (4) found at Kailua that "as long as the torpedo was dipped in corn oil, it made no difference whether the wheat contained corn oil or not." Field experiments were then installed to check Doty's findings, with the following results:.

## EXPERIMENT NO. 5—FIELD 5W HM.—96 STATIONS

	1 torpedo of each kind per station			
	Effective Take—Per Cent			
	2½ days	5½ days	7½ days	9½ days
Standard Wheat no oil.....	3.1	3.0	3.0	3.1
Wheat—Corn oil outside only.....	17.7	21.0	21.2	20.4
Wheat—Corn oil inside and out.....	22.9	33.0	34.3	34.7
Barley—Corn oil outside only.....	12.5	18.0	20.2	20.4
Barley—Corn oil inside and out.....	30.2	35.0	42.4	40.8

A marked increase in effective take is shown for "corn oil inside and out" over "corn oil outside only." Rolled barley was preferred to wheat where the grain was saturated with corn oil but there was no significant difference between wheat and barley with "corn oil outside only."

## RAW LINSEED OIL VS. CORN OIL — BARLEY VS. WHEAT

To compare raw linseed oil with corn oil, and whole wheat with rolled barley torpedoes the following experiment was installed:

## EXPERIMENT NO. 6—FIELD 5 HM.—73 STATIONS

	1 torpedo of each type per station			
	Effective Take—Per Cent			
	1½ days	3½ days	5½ days	11 days
Wheat—Raw linseed oil inside and out.....	35.6	41.1	45.1	47.8
Barley—Raw linseed oil inside and out.....	34.2	50.7	52.1	79.1
Wheat—Raw linseed oil outside only.....	30.1	37.0	38.0	37.3
Barley—Raw linseed oil outside only.....	19.2	24.7	28.2	32.8
Wheat—Corn oil inside and out.....	19.2	24.7	23.9	31.3
Barley—Corn oil inside and out.....	28.8	37.0	40.8	47.8
Wheat—Corn oil outside only.....	11.0	11.0	12.7	17.9
Barley—Corn oil outside only.....	12.3	12.3	9.9	16.4
Wheat, no oil.....	8.2	12.3	11.3	11.9

Under the conditions of this test the rats showed an outstanding preference for "barley-raw linseed oil inside and out."

It is interesting to note that wheat has the edge over barley when the oil is outside only, but that barley is far superior to wheat when the oil is inside and out. The same trend exists in Experiment No. 5.

Both Experiments No. 5 and No. 6 might be classed as "skirmish" tests or "feeler" tests because of the numerous variables per station. The effectiveness of the various torpedoes might be quite different in a test where one station contained several of only one type of torpedo. In these two tests the rats not only had a choice between the various baits but having been attracted by an oil they may have eaten several torpedoes regardless of type to satisfy their hunger. The two tests, however, gave very good indications as to what baits were preferred thus eliminating those which we might class as "not so good," and permitting a better test of fewer variables.

Experiment No. 7 which is summarized below, was planned to compare: wheat torpedoes not treated with oil (the standard torpedo of past years) with "wheat-

corn oil outside only" (our recently declared standard) with "wheat\*-raw linseed oil inside and out" (the second most effective torpedo type of Experiment No. 6). Two torpedoes of one type only were placed in a station to eliminate the possible effect on take that one torpedo type may have on another.

#### EXPERIMENT NO. 7—FIELD 1W AND 5W HM.—93 STATIONS

	2 torpedoes of one type per station	
	Effective Take—Per Cent	
	5½ days	9½ days
Wheat—Raw linseed oil inside and out.....	37.1	47.8
Wheat—Corn oil outside only.....	31.7	48.9
Wheat, no oil.....	7.5	10.8

The results show no difference, under the conditions of this test, between "wheat—raw linseed oil inside and out" and "wheat—corn oil outside only." The very poor effective take of the old wheat standard (no oil) is quite apparent.

Experiment No. 10 was designed to study "barley—raw linseed oil inside and out" (outstanding in Experiment No. 6 in comparison with "barley—raw linseed oil outside only.")

#### EXPERIMENT NO. 10—FIELDS 18 L, 9 HM, AND 30 M—131 STATIONS

	2 torpedoes of one type per station	
	Effective Take—Per Cent	
	3 days*	
Barley—Raw linseed oil inside and out.....	86	
Barley—Raw linseed oil outside only.....	69	

\* Heavy rains washed out many torpedoes and prevented further counts.

The results of this test show a good increase in take for "barley—raw linseed oil inside and out" over the "barley—raw linseed oil outside only."

Based on the data collected to date the plantation practice was changed from "wheat—corn oil outside only" to "barley—raw linseed oil outside only."

#### EFFECTS OF PUTTING OIL INSIDE PARAFFIN-DIPPED TORPEDOES

Observation has shown that when grain is soaked with oil and made up into torpedoes the paper generally becomes impregnated with oil and as a result the hot paraffin, used for water proofing, as well as strengthening, passes into the torpedo.† This is an undesirable feature because paraffin is undigestible and grain coated with paraffin may pass through the system of a rat without effect. The use of an inexpensive but strong wax paper for wrapping will eliminate this problem and at the same time result in reduced costs.

Before standardizing on "barley—raw linseed oil inside and out," the following questions remained to be answered:

\* No thallium sulphate treated barley on hand.

† It has been noticed in using cocoanut oil that the paraffin enters the torpedo where the paper is oil impregnated but flakes off the grain rather than adhering to it. By carefully limiting the oil to 1 pint per 5 pounds of grain and at the same time using 3 sheets of tissue per torpedo the entrance of paraffin is cut to a minimum.

- (1) What effect, if any, does the inward passage of paraffin have on the killing effectiveness of torpedoes?
- (2) Is the killing effectiveness of grain altered by an oil coating?

To answer these questions cage experiments were conducted. A total of 12 healthy field rats (6 large and 6 medium) housed in separate cells were each given  $\frac{1}{2}$  of a "barley torpedo-raw linseed oil inside and out." The oil-treated bait containing the usual quantity of paraffin which passes through oil-impregnated paper proved quite effective with the killing, accompanied by symptoms of thallium sulphate poisoning, of 5 of the 6 medium and 4 of 6 large sized rats.\* Shortly after the completion of this experiment the Pacific Guano and Fertilizer Company increased the thallium sulphate concentration of rolled barley. The experiment was duplicated and every rat died of thallium poisoning.

To saturate the poisoned rolled barley with raw linseed oil before making up torpedoes and then dipping in raw linseed oil would be quite worthwhile as compared to dipping alone, but other grains might be superior to rolled barley and other oils superior to raw linseed oil. Before making further changes in our standard practice it was decided to carry on further tests, to try all likely grains and oils and then select the best as a standard practice.

#### RAW LINSEED OIL VS. COCOANUT OIL

Experiment No. 16 compares poisoned "barley-cocoanut oil outside only" with poisoned "barley-raw linseed oil outside only." Two torpedoes of each type were placed in alternate stations to eliminate the attraction effect that one torpedo type might have on another. The test of 190 stations was laid out in three localities. In making counts, all torpedoes except those untouched were replaced. The results are summarized as follows:

#### EXPERIMENT NO. 16—FIELD 18 L—66 STATIONS

	Effective 2 days	Take—Per 5 days	Cent 8 days
Barley—Cocoanut oil outside only.....	65.2	80.3	71.2
Barley—Raw linseed oil outside only.....	86.4	84.8	72.7

#### FIELD 30 M—88 STATIONS

	Effective 2 days	Take—Per 5 days	Cent
Barley—Cocoanut oil outside only.....	76.1	9.1	
Barley—Raw linseed oil outside only.....	45.5	3.4	

#### FIELD 5 HM—36 STATIONS

	Effective 2 days	Take—Per 5 days	Cent
Barley—Cocoanut oil outside only.....	22.2	11.1	
Barley—Raw linseed oil outside only.....	19.4	0	

\* Thallium sulphate concentration of 1 to 500. Refer to page 159.

## GRAND SUMMARY—190 STATIONS

	Effective Take—Per Cent	
	2 days	5 days
Barley—Cocoanut oil outside only.....	62.1	34.2
Barley—Raw linseed oil outside only.....	54.7	31.1

The extent of the decrease of per cent effective take from the first observation to the second, etc., is an indication of extent of rat population in the area concerned and may be used as a good indication of poison bait preference. The rat population in field 18 L, for example, is known to be unusually large both through observation on the present damage in the field and by the number of rats caught during past harvests in that field and in adjacent fields. The torpedoes set out each time were not enough to satisfy the rat population of the immediate area with the result that the per cent effective take remained very high for the three counts. In fields 30 Makee and 5 Hm. the rat populations are known to be small, particularly in the immediate areas of the tests, and the first sets of torpedoes were sufficient to kill most of the rats. In consequence all but a few of the replaced torpedoes were untouched and the second count showed a marked decrease in effective take for all torpedoes. In field 18 Lihue there were too many rats per bait to allow complete selection. Had the test been carried on for several more counts a point would have been reached with the killing off of the population, where there would be enough baits per rat to allow selection. Unfortunately a scarcity of time prevented further counts in this field. In field 30 Makee and 5 Hm., where the rat populations are small, bait selection was possible and probably occurred. Following this line of reasoning, the true value of the various types of torpedoes must be learned from experiments of this nature where all torpedoes are replaced at each count, and the sum total of effective take of all observations for each type of torpedo is a far more accurate indication of a torpedo's worth than the effective take of individual observations. The following table summarizes this experiment accordingly:

## GRAND SUMMARY

	Total Torpedoes Effective
	5 days
Barley—Cocoanut oil outside only.....	183
Barley—Raw linseed oil outside only .....	162

This summary shows a questionable preference for "barley-cocoanut oil outside only," to "barley-raw linseed oil outside only." The difference is too small to be significant because of the probable error involved in the count and in the effectiveness of the torpedoes missing. Perhaps the surest way of arriving at rat preference for the two oils used in this test is to study the total number of torpedoes untouched.

	Torpedoes Untouched
Barley—Cocoanut oil outside only.....	77
Barley—Raw linseed oil outside only .....	72

Here again the difference is too small to be significant. It might be concluded,

then, that under the conditions of this test, cocoanut oil is just as attractive to rats as raw linseed oil.

BARLEY-COCOANUT OIL INSIDE AND OUT  
vs.  
BARLEY-COCOANUT OIL OUTSIDE ONLY

With Experiment No. 16 showing that cocoanut oil appeals to rats to about the same extent as raw linseed oil, the next step was to see if the effective take could be increased by soaking barley in cocoanut oil before making up the torpedoes and dipping. Cocoanut oil is less expensive than raw linseed oil— a fact of interest to all.

Experiment No. 17 compares “barley-cocoanut oil inside and out” with “barley-cocoanut oil outside only.” Two torpedoes of one type were placed in alternate stations. The test including 310 stations was laid out in three different places. In making counts, all torpedoes except those untouched were replaced. The results are tabulated below :

EXPERIMENT NO. 17—FIELD 30 M—94 STATIONS

	Effective Take—Per Cent	
	3 days	6 days
Barley—Cocoanut oil outside only.....	42.6	25.5
Barley—Cocoanut oil inside and out.....	61.7	56.4

FIELD 4W HM.—68 STATIONS

	Effective Take—Per Cent	
	3 days	6 days
Barley—Cocoanut oil outside only.....	27.9	13.2
Barley—Cocoanut oil inside and out.....	35.3	13.2

FIELD 1W HM.—148 STATIONS

	Effective Take—Per Cent	
	3 days	6 days
Barley—Cocoanut oil outside only.....	28.4	22.3
Barley—Cocoanut oil inside and out.....	33.1	31.1

GRAND SUMMARY

	Effective Take—Per Cent	
	3 days	6 days
Barley—Cocoanut oil outside only.....	32.6	21.3
Barley—Cocoanut oil inside and out.....	42.3	34.8

The results show a significant increase in effective take for “barley-cocoanut oil inside and out.” The significance of the results is brought out very clearly in a study of the total number of torpedoes effective during the 6-day period :

GRAND SUMMARY

	Total Torpedoes Effective 6 days
Barley—Cocoanut oil outside only.....	167
Barley—Cocoanut oil inside and out.....	239



## ANOTHER SKIRMISH TEST

In order to study rat preference for oils in combination and various prospective balanced diets, this skirmish test was installed. The torpedoes were grouped in such a manner as to allow, at the same time, a study of rat preference for the "outside" oils used.

The test was installed in field 30 Makee and one group (three torpedoes of each type) was placed per station, that is: three baits of each type of group A were placed in Station 1, and three baits of each type of group B were placed in Station 2, etc. A total of 224 stations were set out 7 feet apart. The observation summarized below was made 4 days later.

Group:		Per Cent	Per Cent
		Effective Take	Untouched
A	Barley—Cocoanut oil inside and out.....	30	49
	Barley—Cocoanut oil inside and raw linseed oil outside.....	41	33
B	Rolled Oats*—Cocoanut oil inside and out.....	49	40
	Rolled Oats*—Cocoanut oil inside and raw linseed oil outside....	68	15
C	Barley(10)†—Coco Meal(2)†—Cocoanut oil( $\frac{1}{2}$ )—Cocoanut oil out- side .....	25	61
	Barley(10)†—Coco Meal(2)†—Cocoanut oil( $\frac{1}{2}$ )—Raw linseed oil outside .....	40	30
D	Rolled Oats(10)—Coco Meal(2)—Coco oil( $\frac{1}{2}$ )—Cocoanut oil out- side .....	53	31
	Rolled Oats(10)—Coco Meal(2)—Coco oil( $\frac{1}{2}$ )—Raw linseed oil outside .....	65	20
E	Barley(7)—Rolled oats(2)—Coco meal(1)—Coco oil( $\frac{1}{2}$ )—Cocoa- nut oil outside.....	35	46
	Barley(7)—Rolled oats(2)—Coco meal(1)—Coco oil( $\frac{1}{2}$ )—Raw lin- seed oil outside.....	48	30
F	Barley(2)—Hamburger(1)—Cocoanut oil outside .....	27	51
	Barley(2)—Hamburger(1)—Raw linseed oil outside.....	32	39
G	Barley—Linseed oil inside—Cocoanut oil outside.....	10	47

\* Not poisoned.

† Parts by volume.

‡ Not extracted.

The results of this test show a marked rat preference for the "cocoanut oil inside-raw linseed oil outside" oil combination. There is also shown an outstanding preference for rolled oats over barley. The rolled oat torpedoes were made up of poison-free grain as no thallium sulphate treated rolled oats were on hand. The lack of poison on the grain may play a large part in the rolled oat preference shown in the results. The test was conducted as a "feeler" with the hope of finding a lead on which to concentrate.

The increase in effective take of rolled oats over rolled barley may be outweighed by the additional cost per unit of grain. A pound of poisoned rolled oats may kill a few more rats than a pound of poisoned barley, but a dollar's worth of poisoned barley may kill more rats than a dollar's worth of poisoned oats.

The barley-hamburger mixture was not particularly attractive to the rats. The paraffin dipping, necessary to prevent breakage, prevented the exit of the meat

odor, and so the torpedoes were dipped, some in cocoanut oil and some in raw linseed oil, to give them the same odor as the other torpedoes being tested.

The results for group "E" indicate that the addition of a small part of rolled oats to the barley bait, "cocoanut oil inside-raw linseed oil outside," may result in a maximum effective take per unit cost.

ROLLED OAT-ROLLED BARLEY PROPORTIONS-COCOANUT OIL INSIDE-RAW LINSEED  
OIL OUTSIDE VS. ROLLED BARLEY-COCOANUT OIL INSIDE-  
RAW LINSEED OIL OUTSIDE

"Barley-cocoanut oil inside-raw linseed oil outside" is preferred to "barley-cocoanut oil inside and out," the bait outstanding until the last "feeler" test. Rolled oats have been shown to be preferred to barley with the possibility of a very desirable barley-rolled oat mixture which if as effective as rolled oats alone would be a more economical bait because of the large difference in the price of the two grains.

Experiment No. 18 was designed to make a final study of the "cocoanut oil inside-raw linseed oil outside" oil combination and to study rolled oat (unpoisoned) and rolled barley proportions. The experiment was installed in three places throughout the Wailua Homestead region. Two torpedoes of one type only were placed in a station. The stations, 680 in number, were marked by fence posts from 4 to 7 feet apart. In making counts, all torpedoes except those untouched, were replaced. The results (all areas combined) are summarized below:

	Effective Take—Per Cent	
	2 days	4 days
Oats—Cocoanut oil inside—linseed oil outside.....	82.9	46.8
Barley—Cocoanut oil inside and out.....	64.7	10.4
Barley—Cocoanut oil inside—linseed oil outside.....	60.6	4.5
Barley(5)*—Oats(1)—Coco oil† inside—linseed oil outside.....	71.2	17.5
Barley(5) —Oats(2)—Coco oil inside—linseed oil outside.....	81.2	32.5
Barley(5) —Oats(3)—Coco oil inside—linseed oil outside.....	81.8	15.6
Barley(5) —Oats(4)—Coco oil inside—linseed oil outside.....	86.5	14.9
Barley(5) —Oats(5)—Coco oil inside—linseed oil outside.....	79.8	21.7

\* Parts by volume.

† Cocoanut oil.

	Total Torpedoes	
	Effective	Untouched
Oats—Cocoanut oil inside—Linseed oil outside.....	213	35
Barley—Cocoanut oil inside and out.....	126	97
Barley—Cocoanut oil inside—Linseed oil outside.....	110	122
Barley(5)*—Oats(1)—Coco oil† inside—linseed oil outside.....	148	81
Barley(5) —Oats(2)—Coco oil inside—linseed oil outside.....	188	41
Barley(5) —Oats(3)—Coco oil inside—linseed oil outside.....	163	72
Barley(5) —Oats(4)—Coco oil inside—linseed oil outside.....	170	72
Barley(5) —Oats(5)—Coco oil inside—linseed oil outside.....	167	60

\* Parts by volume.

† Cocoanut oil.

The results of this test show a rat preference for the rolled oat bait with the "barley (5)-oats (2)" mixture a close second. A study of the total torpedoes

effective and those untouched shows these baits greatly preferred to the others included in the test. The "barley (5)-oat (2)" mixture is the less expensive of the two and may account for just as many dead rats per unit cost. The rats and mice of this locality show a slight preference for the "barley-cocoanut oil inside and out" over the "barley-cocoanut oil inside-raw linseed oil outside."

ROLLED BARLEY (5) ROLLED OATS (2) COCOANUT OIL INSIDE RAW LINSEED OIL OUTSIDE VS. VARIOUS MEAT BAITS (SAUSAGE)

Various meat baits (sausage), prepared by Garlough, have been used in the pineapple fields of Lanai with very good success. Four types of Garlough's baits containing specific amounts of fresh hamburger, cracked barley alone or in combination with ground copra, thallium sulphate and macadamia nut oil, and packed in sausage skins, were set out in a test for a comparison with one of the outstanding grain baits, "rolled barley (5)-rolled oats (2) cocoanut oil inside-raw linseed oil outside." A total of 180 baits of each type were set out; two of one type only per station. Bait replacements were not made. The results of the test are summarized below:

EXPERIMENT NO. 19—FIELD 30 MAKEE—450 STATIONS

	Effective Take—Per Cent	
	24 hours	3 days
Meat bait No. 114.....	31	44
Meat bait No. 117.....	55	66
Meat bait No. 118.....	40	52
Meat bait No. 119.....	37	48
Lihue grain bait.....	30	66

The meat baits were comparatively small and also darker in color than the grain bait and in consequence they were very difficult to relocate. In any number of cases they were carried away and partly eaten by ants, roaches and other insects. Many baits reported missing at the first count were found during the second count. The per cent effective takes for the meat baits are not absolutely true because of the large probable error involved. The grain torpedoes, on the other hand, are large and white and consequently easy to find and are too heavy to be moved by ants, roaches and other insects. The effective take for the grain torpedoes, therefore, is very accurate—the probable error small.

A study of the number of torpedoes untouched after 3 days' exposure is interesting:

	Total Torpedoes Untouched
Meat bait No. 114.....	100
Meat bait No. 117.....	62
Meat bait No. 118.....	85
Meat bait No. 119.....	93
Lihue grain bait.....	2

INCREASED THALLIUM SULPHATE CONCENTRATION FOR MAXIMUM KILL

While these poison bait experiments were being conducted it was noticed in observations 18 to 24 hours after setting out the improved oiled grain torpedoes

that the rats and mice had done a great deal of nibbling, had chewed many oily paper tops, and had eaten torpedoes to the extent of 25 per cent and more. The largest effective take, however, occurred during the 24 to 48 hour period. Did the rat that ate 25 per cent the first night come back for more the second night? If not, the rat may have been large enough and strong enough to pass off the poison contained in its small meal of  $\frac{1}{4}$  torpedo. Had the 25 per cent eaten torpedoes been effective in Experiment No. 19 the per cent effective take for the Lihue grain bait would have been 86 per cent instead of 66 per cent and very small rats and mice would have been killed by fair-sized nibbles.

The efficiency of the newer baits can only reach a maximum by increasing the thallium sulphate concentration to about one pound per 100 pounds of grain. A quarter of a torpedo would then be a lethal dose for all rats, and fair-sized nibbles would kill small-sized rats and mice. Thallium sulphate is very expensive and the increase in kill may not be large enough to offset the additional cost. Whether or not the additional thallium sulphate would have any effect on the attractiveness of the bait remains to be determined.

The plantation practice of "rolled barley-raw linseed oil outside only" has been changed to "rolled barley-cocoanut oil inside-raw linseed oil outside." Effective take per unit cost of the better baits is being investigated and the bait which gives the greatest kill per dollar will be adopted as a standard at Lihue.

#### SUMMARY

A rat does damage to the extent of at least two dollars to sugar cane in the course of a year.

The rat problem at The Lihue Plantation Company, Limited, is discussed.

The value of catching rats in the harvest fields is pointed out.

"Effective take" of poison baits is discussed.

The acceptance of whole wheat torpedoes is greatly increased by corn oil dipping.

Corn oil dipping was adopted as a standard practice at Lihue.

"Whole wheat torpedoes-corn oil inside and out" are preferred by rats to "whole wheat torpedoes-corn oil outside only."

Rats prefer rolled barley to whole wheat.

Rats do not eat the hulls of rolled barley.

Rolled barley is 33 per cent lighter than wheat per unit volume.

"Rolled barley torpedoes-corn oil inside and out," are preferred by rats to "whole wheat torpedoes-corn oil inside and out."

Rats prefer raw linseed oil to corn oil.

The Lihue practice was changed to "rolled barley-raw linseed oil outside."

Where either corn oil or raw linseed oil is outside only, wheat torpedoes tend to be more attractive to rats than barley torpedoes but where either oil is inside and out, barley is much preferred to wheat.

"Rolled barley-raw linseed oil inside and out" is preferred by rats to both "rolled barley-corn oil inside and out" and "rolled barley-raw linseed oil outside only."

"Rolled barley-cocoanut oil outside only" is equally as attractive to rats as

"rolled barley-raw linseed oil outside only." Coconut oil is less expensive.

"Rolled barley-coconut oil inside and out" is greatly preferred by rats to "rolled barley-coconut oil outside only."

"Rolled barley-coconut oil inside-raw linseed oil outside" is preferred by rats to both "rolled barley-coconut oil inside and out," and to "rolled barley-raw linseed oil inside-coconut oil outside."

The plantation practice was changed to "rolled barley-coconut oil inside-raw linseed oil outside."

"Rolled oats-coconut oil inside-raw linseed oil outside" is preferred by rats to "rolled barley coconut oil inside-raw linseed oil outside."

A mixture of "5 parts of rolled barley-2 parts of rolled oats-coconut oil inside-raw linseed oil outside" is nearly as attractive to rats as "rolled oats alone-coconut oil inside-raw linseed oil outside." These two baits are outstanding and so nearly the same in rat acceptance that effectiveness per unit cost must be the deciding factor.

Under the conditions of field 30 M, the "barley(5)-oats(2) mixture-coconut oil inside-raw linseed oil outside" proved superior to various meat baits prepared by Garlough.

Hot paraffin passes through oil-impregnated wrapping tissue. Where raw linseed oil coats the grain, the paraffin adheres to the grain; but where coconut oil coats the grain the paraffin does not adhere, but flakes off. The entrance into a torpedo of hot paraffin can be reduced to a minimum by limiting the inside oil to one pint to 5 pounds of grain (rolled barley) and by using 3 sheets of wrapping tissue per torpedo. The use of an inexpensive but strong wax paper may be the solution to this problem.

The coating of grain with oil does not alter the killing effectiveness of the poisoned grain.

The efficiency of the newer baits can only reach a maximum by increasing the thallium sulphate concentration to about one pound per 100 pounds of grain. A quarter of a torpedo would then be a lethal dose for any rat, and fair-sized nibbles would kill small-sized rats and mice. Because of the high cost of thallium sulphate the increase in kill may not be large enough to offset the additional cost. Whether or not the additional thallium sulphate would have any effect on the attractiveness of the bait remains to be determined.

Effective take per unit cost of the better baits is being investigated. The bait which gives the greatest kill per dollar will be adopted as a standard at Lihue.

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## Yams for Hawaiian Gardens

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By E. L. CAUM and J. P. MARTIN

In the spring of 1935 several tubers of an unidentified Oriental yam were obtained from the Philippines by Dr. H. L. Lyon. To check its value as an accessory food crop for the plantations, and also to permit its identification, plantings were made at the Pathology Plot of the Experiment Station. It proved to be the Spiny yam or, as it is known in the Straits Settlements, the Lesser yam, *Dioscorea esculenta*. The plant is a native of some part of the eastern Asiatic region, occurring in the wild in India, Malaya, the Philippines and Guam, although there is no definite evidence as to just where the species actually originated. The tubers of this yam are of considerable economic importance as a food crop, and as a result the plant has been transported far and wide throughout the Asiatic tropics. It has not, however, attained the range of another important Asiatic yam, *Dioscorea alata*, the Winged or Greater yam, which is now cultivated throughout the tropics the world around.

As a natural result of its wide distribution and long cultivation, the Spiny yam is distinctly variable in a number of characters, such as size, shape and flavor of the tubers, the size of the leaves, the degree of spininess of the stems and roots and the ability to produce flowers and seeds. This variability and spontaneous occurrence in many places are reflected in the multiplicity of names, both vernacular and technical, by which the plant is known.

The earliest reference to this yam that we have found is in Vol. 5 of Rumphius' *Herbarium Amboinense* (1750) where, on pages 357-359, under the name Combili or Combilium, three varieties are discussed at length in Latin and Dutch, and illustrated with a full-page plate, Tab. 126, which is here reproduced as Fig. 1. The first technical description of the species was published in 1790 when Loureiro in his *Flora Cochinchinensis* named it *Oncus esculentus*. Later authors recognized the plant as a species of *Dioscorea*, but apparently did not recognize the identity of their specimens with the *Oncus esculentus* of Loureiro. Thus throughout the literature we find the plant described many times under many names, by Roxburgh three times, as *Dioscorea aculeata*, *D. fasciculata* and *D. spinosa*, by Kunth as *D. tiliaefolia*, by Hemsley as *D. cymosula*, by Balfour as *D. lanata*, by Blanco as *D. sativa* and *D. tugai*, to list only a few. The identity of the plants to which these names were applied with the one described by Loureiro was recognized by Burkill in an article in *The Gardens' Bulletin of the Straits Settlements*, Vol. 1 (1917) pp. 396-399. The correct scientific name thus becomes *Dioscorea esculenta* (Loureiro) Burkill.

The plant is a twining vine, growing to a length of probably 20 to 30 feet in the usual crop period of 8 to 9 months. The stems are round in cross-section, slightly tomentose, especially on the young growth, and armed, particularly near the base, with small sharp thorns. There are always two larger hooked spines at the base of each leaf stalk. The leaves are heart-shaped, up to six inches in diameter or even more, with prominent veins (Fig. 2). The flowers are green, about one-sixth inch in diameter, and are borne in long slender drooping spikes which arise, usually singly,

in the axils of the leaves. Flowers are rarely formed, however, male flowers even more rarely than female. The tubers are predominately roughly cylindrical to spindle- or carrot-shaped with an appreciable percentage of lobed or toed forms, and covered with many very short rather stiff fibrous rootlets (Fig. 3). The flesh is white, the thin skin light brown. From the base of the stem there arise three classes or categories of underground organs (Fig. 4). First there are the smooth rather heavy cord-like structures of various lengths, on the ends of which the tubers are formed. These tend to extend straight out or slightly upward, and the tubers themselves frequently point upward so that they, or at least their free ends, lie very near the surface of the ground. It is for this reason that, in cultivation, the plants are hilled up. The second class of organs arising from the common center are the feeding roots, long, slender and sparsely branched. Last, but by no means least, are the protective organs. These, possibly modified feeding roots, are rather heavy stiff structures bearing formidable spines often an inch in length (Figs. 4 and 5). They form a veritable barbed wire entanglement around the feeding roots and some of the tubers, and furnish the incentive for the name, the "Barbed Wire yam," which the plant has acquired at the Experiment Station. It is difficult to see just what the significance of these thorny structures may be, as they are not sufficiently long to enclose all the tubers, most of which lie well beyond their protection. In fact the tubers, lying close to the surface of the soil, seem to invite the attention of pigs. The main function which might be ascribed to the underground thorns is to interfere with the human harvesting of the crop. Despite the necessity of contending with these spikes the yam is extensively cultivated throughout the Oriental tropics, and in areas where the plant grows spontaneously, tubers of the wild plants are dug and gathered in the jungle.

The tubers furnished by Dr. Lyon were used for planting material, and on April 10, 1935, twenty-two cuttings were planted in two rows at the Pathology Plot, the rows and the cuttings spaced approximately two feet apart. The preparation of the seed pieces is simple, the yam tubers being handled exactly like the tubers of the ordinary white potato. In common with the fleshy storage roots or underground branches of many other plants, the yams have the faculty of developing adventitious buds at almost any point on the exterior. Six of the cuttings failed to grow, and the sixteen vines resulting from the others were trained over a rough bamboo trellis (Fig. 6), and the plants hilled up during their growth. After nine months, (on January 9, 1936, to be exact), when the leaves had begun to turn yellow and fall, the vines were torn down and the tubers harvested. No fertilizer was applied, as no symptoms of plant food deficiency were noted at any time during the growth of the vines. The tubers obtained from these plants varied greatly in size and shape, ranging upwards from tiny nubbins about the size of a marble. The largest one, shown in Fig. 7, weighed four pounds. The normal type were from 8 to 10 inches in length and 6 to 8 inches in circumference, with a weight of about  $1\frac{1}{2}$  to 2 pounds, which seems to be about the average size for tubers of this species. The total yield from the sixteen hills (Fig. 8) weighed 151 pounds.

In the article by Burkill, previously referred to, the tubers of a number of races from India, Indo-china and the Philippines are described and illustrated, but the

particular form which we have here does not seem to be among those discussed. At least, the tubers of our plant do not correspond especially well with any of those figured, although they resemble Burkill's number 276, a Philippine race, more closely than any of the others.

We are not in position to say much about the preparation of the yams for the table, except that they should be peeled and boiled for twenty minutes or so, to remove a crystalline substance similar to the calcium oxalate in taro, and a bitter principle that is sometimes present. After boiling they may be served direct, or baked if desired. According to the members of the Experiment Station staff who have sampled them, the yams are decidedly palatable, and there was some slight difficulty experienced in preventing the Filipino helpers at the Station from making away with the entire crop. There is apparently no reference to methods of preparation of these vegetables in any of the literature available.

In a survey of the markets in Honolulu we have found four kinds of locally grown yams offered for sale (Figs. 9-12) and one kind imported from China (Fig. 13). At least one other Chinese variety has been seen in the past, but no tubers were found in the markets at this time. In addition to these true yams, locally grown tubers of the Yam Bean, *Pachyrrhizus erosus* (Fig. 14) are sold as yams, and the heavy tuberous roots of an unidentified plant, possibly a Composite (Fig. 15), are imported as yams from China. Tubers of the Spiny yam were not found in the markets.

Shortly after this market survey, we received as a gift from the Bishop Museum a single tuber (Fig. 16) of a giant yam that was brought to Honolulu from Fiji. This tuber weighed 31 pounds. It is probably the same variety that is grown throughout southern and southeastern Polynesia, where it is said to produce tubers up to 50 pounds or even more in weight.

Further plantings of the Spiny yams will be made, as well as of the other varieties which we now have or which may later be obtained, in order to obtain sufficient planting material to supply any of the plantations that may wish to make an undoubtedly popular addition to their vegetable gardens; more particularly those connected with the Filipino camps.





Fig. 1. The Spiny yam, from the *Herbarium Amboinense*.

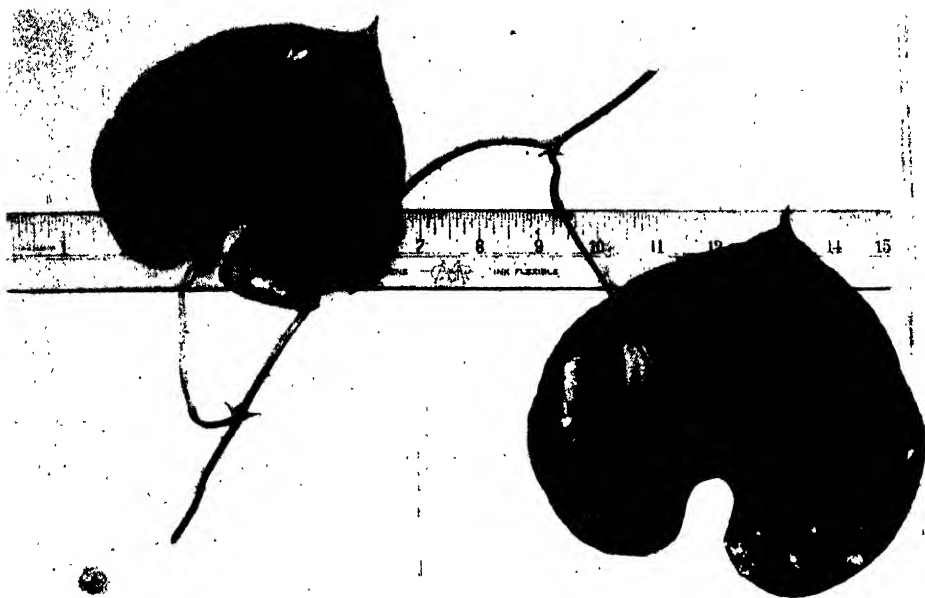


Fig. 2. The leaves and stem of the Spiny yam, showing the hooked spines at the base of the petioles.



Fig. 3. Tubers of the Spiny yam.

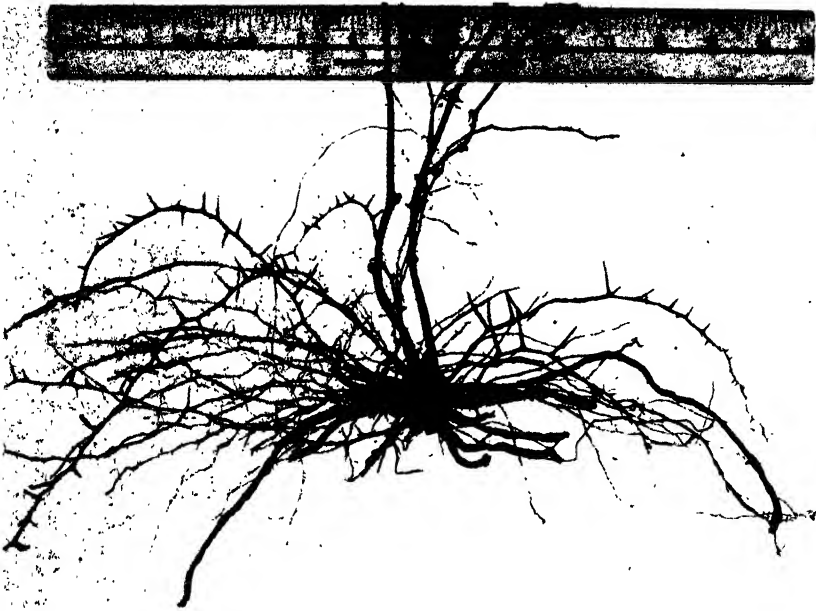


Fig. 4. The base of the stem and the underground parts of the plant, showing the stubs of the tuber stalks, the feeding roots and the protective roots.

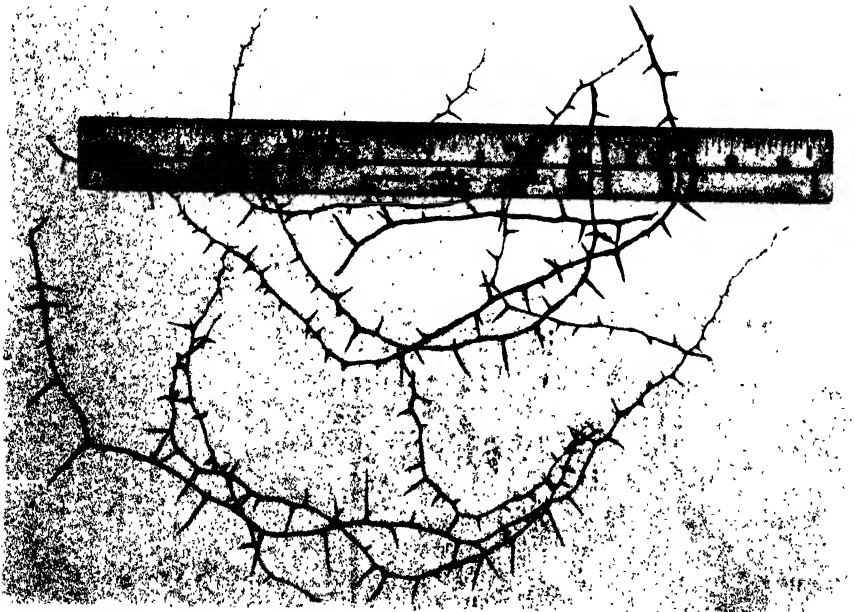


Fig. 5. Several of the protective roots, detached from the plant.



Fig. 6. The vines at the Pathology Plot, just before harvesting.

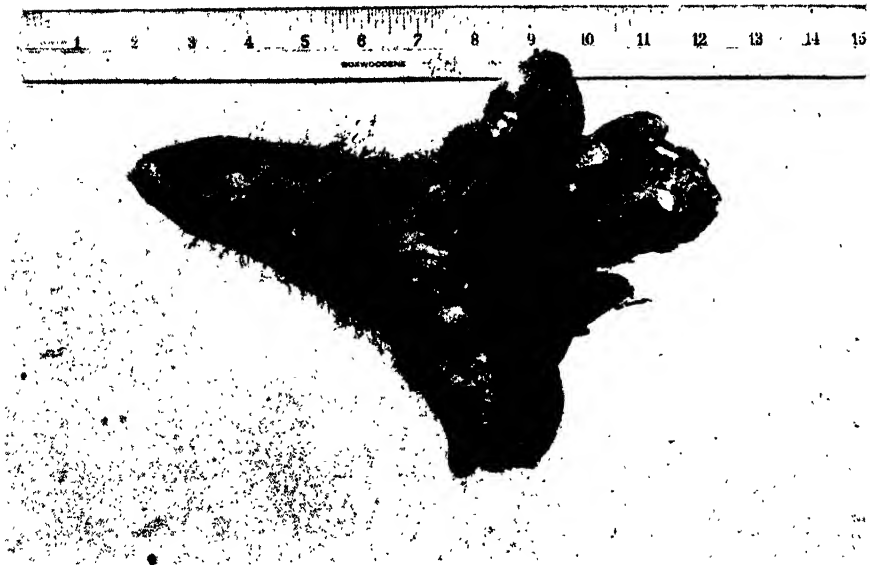


Fig. 7. The largest single tuber in the crop.

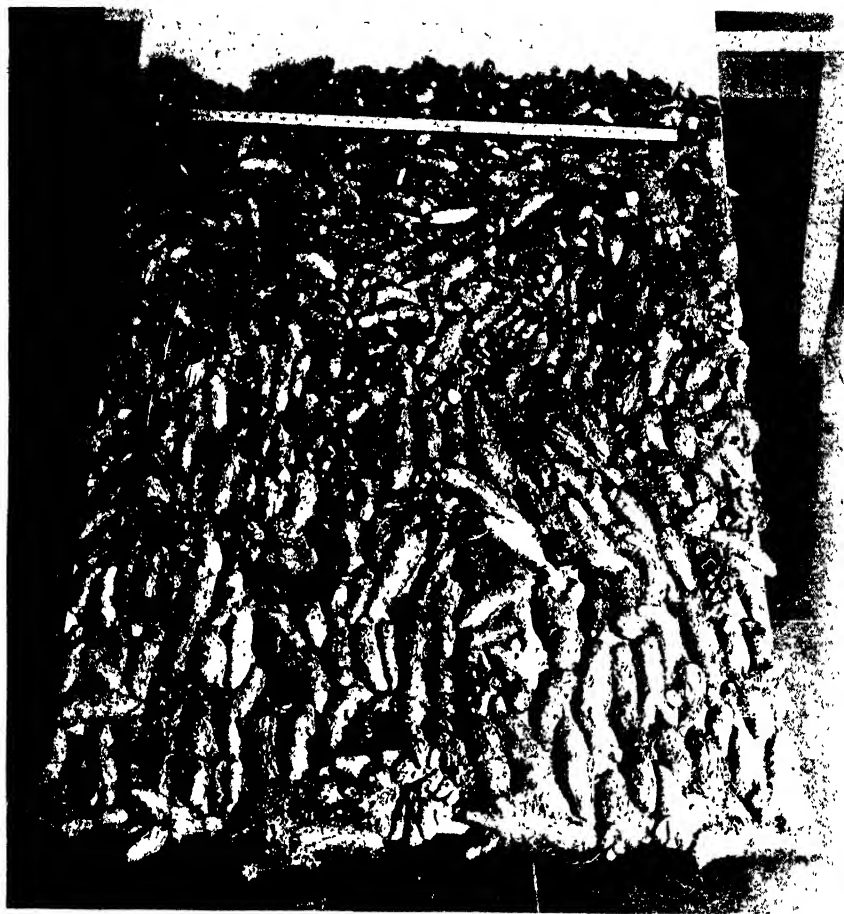


Fig. 8. One hundred and fifty-one pounds of yams from sixteen vines. The measure is a meter stick,  $39\frac{1}{8}$  inches.

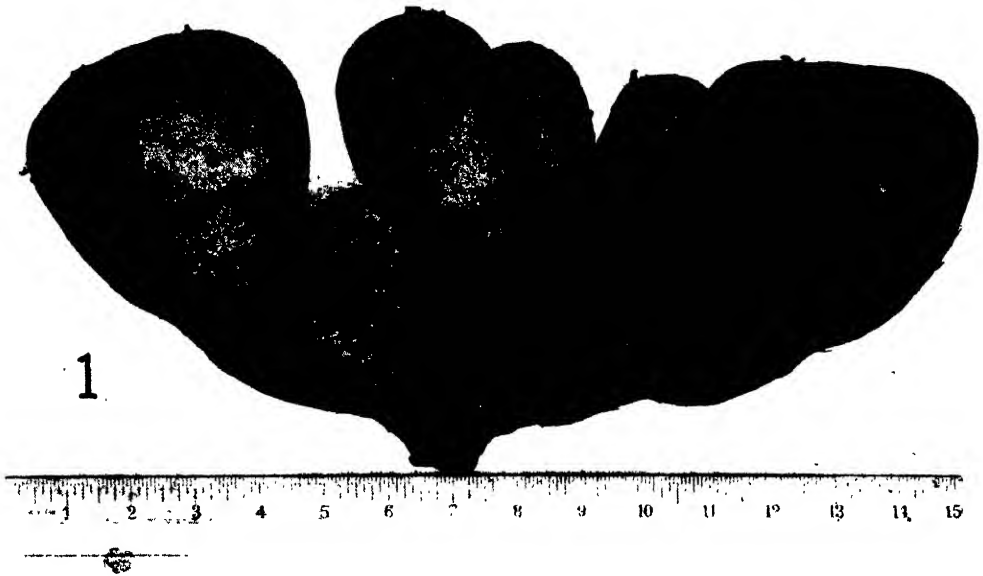


Fig. 9. The tuber of a locally grown species of yam, from the market.



Fig. 10. Tubers of a locally grown species of yam, from the market.

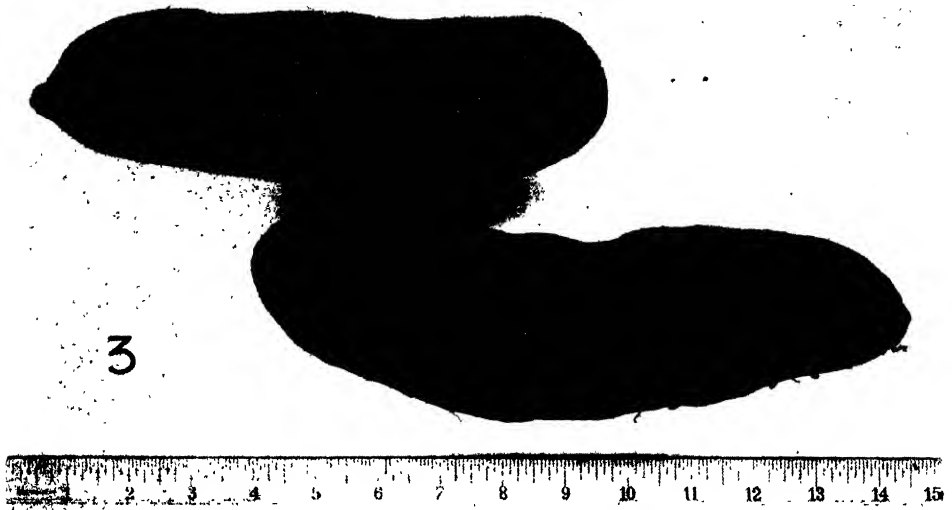


Fig. 11. Tubers of a locally grown species of yam, from the market.

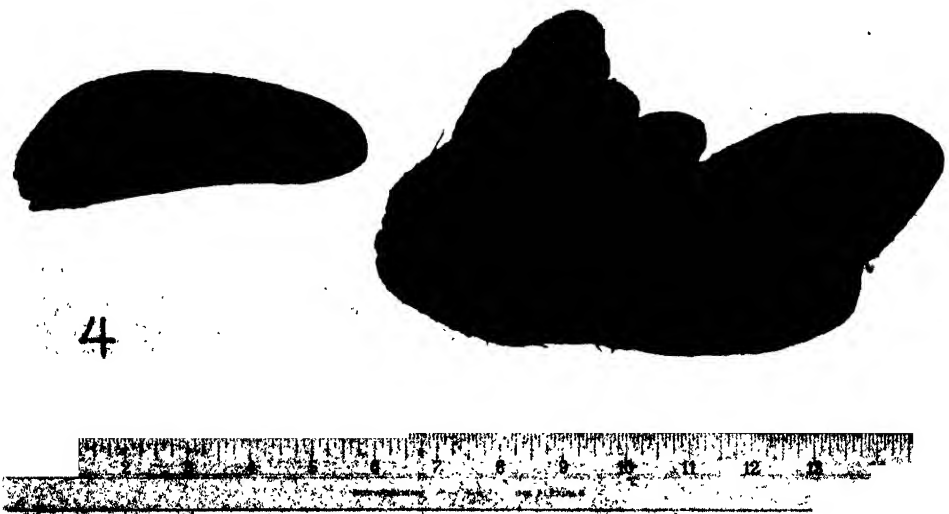


Fig. 12. Tubers of a locally grown species of yam, from the market.



Fig. 13. Tubers of a species of yam imported from China, from the market.

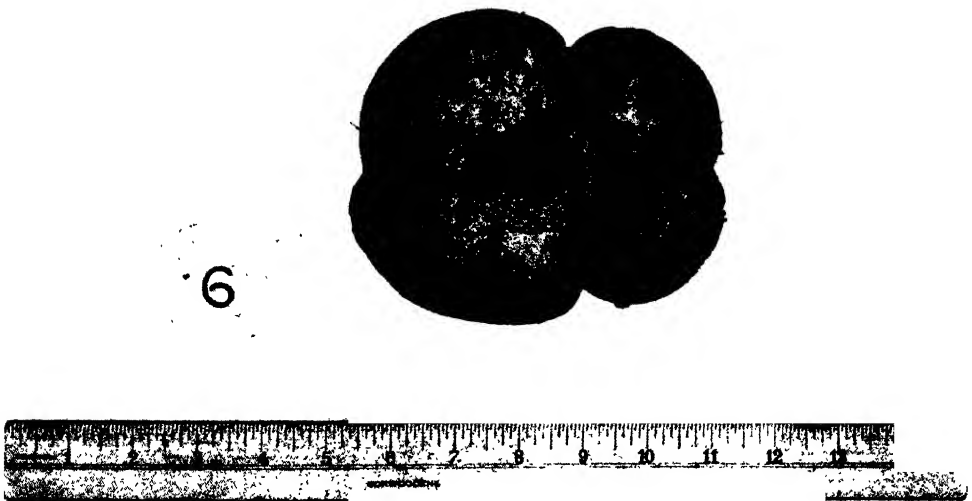


Fig. 14. The tuber of the Yam Bean, from the market.





Fig. 15. Tuberous roots of an unidentified Chinese plant, sold as yams, from the market.



Fig. 16. A tuber of a giant Fijian yam.

## Primary and Total Combustion Volume in Factory Boilers

---

The committee, consisting of G. H. W. Barnhart, R. B. Johnson and R. H. Lloyd, appointed at the 1932 Annual Meeting of the Association of Hawaiian Sugar Technologists for defining combustion volume measurements, gave us the following definitions which we have requested be followed in reporting combustion volume in the Synopsis schedules.

*Primary Combustion Volume:* Include the cubical contents of the furnace immediately above the fuel bed, assuming that the fuel bed covers the grates and adjacent shelves (where installed) or the floor in case of a Cook furnace.

*Total Combustion Volume:* Include the cubical contents of the furnace between the grate, or floor in case of a Cook furnace, and the first place of entry into or between the tubes as indicated by any one of sketches Nos. 1 to 7, pages 159 to 161, of the 1932 Report of Association of Hawaiian Sugar Technologists.

The spaces reported should be those intended for combustion of bagasse. If whole or parts of furnaces are intended for oil burning, such space should be reported separately. An illustration of this distinction is given in Fig. 7, page 161, of the 1932 Report noted above.

SUGAR TECHNOLOGY DEPARTMENT.

W. L. M.

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## Mill Roller Openings

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The report of the committee on Mill Opening Measurements of the Association of Hawaiian Sugar Technologists to recommend a standard method of expressing mill openings for use in the Annual Synopsis was presented by A. S. Taylor at the 1934 Annual Meeting of the Technologists and adopted October 23, 1934. We have requested that the recommendations be followed in preparing Synopsis schedules. Their report and examples follow:

Your committee appointed to recommend a standard method for expressing mill openings begs to report as follows:

Measurement referred to hereafter shall be the dimension from the tip to the bottom of the groove, representing the vertical distance between these points.

We recommend that mill opening be expressed in the following form:

T (O) B

when:

T = Distance from tip to bottom of groove of top roller.

B = Distance from tip to bottom of groove of bottom roller.

O = Vertical distance, plus or minus, from tips of respective rollers. A minus dimension shall indicate that the rollers are in mesh and overlap. When "O" is plus, this is the actual measurement between tips of the top and bottom roller, measured vertically.

*Examples:*

1. When the grooves are  $\frac{3}{4}$ " deep in both top and bottom rollers, and the tips are  $\frac{1}{4}$ " apart, the opening would be written:

$\frac{3}{4}$ " (+  $\frac{1}{4}$ ")  $\frac{3}{4}$ "

2. When the grooves are  $\frac{3}{4}$ " deep in both rollers, and the tips of both rollers are exactly in line, the opening would be written:  
 $\frac{3}{4}$ " (o)  $\frac{3}{4}$ "
3. When the grooves are  $\frac{3}{4}$ " deep in both rollers, and the tips overlap  $\frac{1}{4}$ ", the opening would be written:  
 $\frac{3}{4}$ " ( $-\frac{1}{4}$ ")  $\frac{3}{4}$ "
4. When the grooves are  $\frac{3}{4}$ " deep in both rollers, and the rollers are set iron to iron, the opening would be written:  
 $\frac{3}{4}$ " ( $-\frac{3}{4}$ ")  $\frac{3}{4}$ "
5. When the grooves are  $\frac{3}{8}$ " in the top, and  $\frac{3}{4}$ " in the bottom roller, and the distance between tips is  $\frac{1}{4}$ ", the opening would be written as follows:  
 $\frac{3}{8}$ " ( $+\frac{1}{4}$ ")  $\frac{3}{4}$ "
6. Under the conditions of 5, but with the grooves meshing and set iron to iron (assuming perfect matching of grooves) the opening would be written:  
 $\frac{3}{8}$ " ( $-\frac{3}{8}$ ")  $\frac{3}{4}$ "

SUGAR TECHNOLOGY DEPARTMENT.

W. L. M.

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## Sugar Prices

96° CENTRIFUGALS FOR THE PERIOD  
DECEMBER 30, 1935, TO MARCH 11, 1936.

Date	Per Pound	Per Ton	Remarks
Dec. 30, 1935.....	3.25¢	\$65.00	Puerto Ricos.
“ 31.....	3.28	65.60	Cubas.
Jan. 7.....	3.10	62.00	Cubas or duty frees.
“ 8.....	3.20	64.00	Puerto Ricos.
“ 9.....	3.15	63.00	Philippines.
“ 13.....	3.20	64.00	Cubas, Puerto Ricos.
“ 14.....	3.19	63.80	Cubas, 3.18, 3.20.
“ 16.....	3.20	64.00	Cubas.
“ 17.....	3.235	64.70	Cubas, 3.22, 3.25; Puerto Ricos, 3.25.
“ 18.....	3.275	65.50	Cubas, 3.25; Philippines, 3.30.
“ 20.....	3.325	66.50	Cubas, 3.32; Puerto Ricos, 3.33.
“ 24.....	3.35	67.00	Puerto Ricos.
“ 27.....	3.38	67.60	Cubas.
“ 29.....	3.35	67.00	Puerto Ricos.
Feb. 5.....	3.30	66.00	Puerto Ricos.
“ 14.....	3.31	66.20	Puerto Ricos, 3.30, 3.32.
“ 15.....	3.30	66.00	Puerto Ricos.
“ 18.....	3.35	67.00	Puerto Ricos.
“ 21.....	3.39	67.80	Puerto Ricos, 3.38; Philippines, 3.38, 3.40.
“ 24.....	3.40	68.00	Puerto Ricos.
“ 28.....	3.45	69.00	Philippines, Puerto Ricos.
Mar. 4.....	3.49	69.80	Cubas.
“ 5.....	3.50	70.00	Cubas.
“ 6.....	3.5367	70.73	Puerto Ricos, 3.50, 3.55; Cubas, 3.56.
“ 11.....	3.51	70.20	Cubas.



# THE HAWAIIAN PLANTERS' RECORD

Vol. XL

THIRD QUARTER, 1936

No. 3

*A quarterly paper devoted to the sugar interests of Hawaii and issued by the Experiment Station for circulation among the plantations of the Hawaiian Sugar Planters' Association.*

## **In This Issue:**

### *Soil and Plant Material Analyses by Rapid Chemical Methods:*

In the fall of 1932 the manager of a sugar plantation on the Island of Kauai became interested in the potential usefulness of certain popular soil testing "kits." His staff conducted an experimental trial and study of two of these kits, finding, in due course, quite satisfactory correlation of "kit" analytical data with corresponding figures secured by more elaborate analytical procedure.

Previous to this the Director of the Agricultural Extension Division of the University of Hawaii had made experimental tests with a number of kits and in these studies he had found them reasonably reliable, and capable, he thought, of improvement and development for more exacting usage.

The ease and rapidity with which "kit" analyses could be made gave rise to the suggestion that plantation workers generally might employ them in making field nutrient soil surveys and adapt them for other analytical purposes in the various problems confronting the plantation staffs.

The popularity of the kits grew rapidly. Other plantation men added them to their experimental equipment. In the summer of 1933 the Experiment Station, H.S.P.A. in Honolulu began a critical study of kit procedures in an effort to correct certain inadequacies of technic which were found to apply more particularly to the more common types of Hawaiian soils.

This article depicts in some detail the circumstances relating to the early pioneering studies of soil testing kits on the plantations and the attempt to modify kit procedures to make them applicable to Hawaiian conditions.

A sketch is presented of the plantation and Experiment Station cooperative plan of soil nutrient survey in the early 1920's. Circumstances are cited which led to the abandonment of the citric soluble survey and to the adoption of rapid chemical methods of soil analysis (in supplanting the citric soluble process).

Descriptions appear in the article bearing upon the researches conducted in the development of new and rapid methods of soil and plant analyses.

Comments are made upon the growth of plantation interest in making soil and plant nutrient studies upon the plantation premises. The development of separate agricultural-chemical laboratories on plantations is discussed together with the co-

operative activities of the Experiment Station in Honolulu in contributing to the progress of this work.

The transition from kit analyses to the more reliable rapid chemical methods (R.C.M.) is described. The coordination of activities in agricultural-chemical studies by workers in the Industry is presented in this paper as the fabric upon which the R.C.M. enterprise has come into being.

Sections of the paper are devoted separately to descriptions and discussions, respectively, of each rapid method of analysis employed and to detailed procedures followed in conducting all R.C.M. studies.

Formulae and instructions are included for preparing and standardizing the reagents employed in the work.

In August 1936 Hawaiian plantation and Experiment Station R.C.M. studies embraced an organization consisting of forty-three individual laboratories and about one hundred fifty analysts, agriculturists, chemists and other workers.

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# Soil and Plant Material Analyses by Rapid Chemical Methods

By FRANCIS E. HANCE

## CONTENTS

	PAGE
Foreword .....	191
The Citric Acid Soluble Project.....	192
Collection of soil samples.....	192
The plantation survey.....	192
The analyses .....	192
The report to the plantation.....	193
Interpretation of citric soluble and associated data.....	193
Reasons for its decline.....	193
The Introduction of Soil-Testing Kits in Hawaii.....	194
Rapid Chemical Analyses Not a New Idea.....	194
Prof. F. G. Krauss and the Extension Service of the University of Hawaii..	194
Grove Farm Investigates Soil Testing Kits.....	195
The Experiment Station Investigates Kit Procedures in the Field.....	195
The First Departure From Conventional Kit Technic.....	195
Experimental Results of Field Trials Using Slightly Modified Kits.....	196
A Laboratory Study of the Original Kits in Honolulu.....	196
Difficulties With the Potash Soil Kit.....	197
Extended Experimental Trial of the Kits on a Few Plantations.....	198
Some Advantages of Original Kit Assemblies.....	198
Other Objectionable Features of Commercial Kits.....	198
A Plantation Staff Cites Its Kit Experiences.....	198
The Hawaiian Soil Phosphate Fixation Kit.....	200
Developments Regarding Departures From Commercial Kit Procedures....	200
The Gradual Evolution From Kit Tests to R.C.M.....	201
Color Standards .....	201
Color Comparison and Turbidity Apparatus.....	201
The Potash Rotator.....	203
The Determination of Phosphate in Soil.....	207
A Central Depot For Reagents and Apparatus.....	208
Classes of Instruction at the Experiment Station for Plantation Analysts....	208
Supplementing R.C.M. Studies of Soil Potash and Phosphate With New Analytical Assemblies Designed to Expand the Field of Plantation Research .....	209
Plantation and Experiment Station Check on Analytical Technic.....	209
Cooperative Features of the R.C.M. Enterprise.....	211
The Organization of R.C.M.....	212
Correlations .....	212
Soil Tests .....	217



A Discussion on Methods of Chemical Analyses.....	218
Available nitrogen in soil.....	219
Ammoniacal nitrogen .....	219
Nitrate nitrogen .....	221
Total nitrogen in cane juice, plant material, mill by-products and soil...	221
Nitrogen in irrigation and mill waters.....	223
The determination of readily soluble phosphate in soil.....	224
The estimation of soil phosphate fixation.....	225
The determination of phosphate in cane juice.....	227
The determination of free or soluble phosphates in boiler water.....	229
The determination of phosphate in filter cake.....	229
The determination of phosphate in mill ash.....	229
The determination of readily soluble potash in soil.....	230
The determination of potash in cane juice.....	231
The determination of potash in irrigation water.....	232
The determination of potash in mill ash.....	232
The determination of potash in molasses.....	233
The determination of readily soluble calcium in soil.....	233
The determination of total calcium in filter cake.....	234
The colorimetric determination of soil reaction (pH).....	234
The Reagents Used in R.C.M. Work.....	235
A word regarding reagents.....	236
Reagent No. 1, $K_2O$ .....	236
Reagent No. 2, $K_2O$ .....	236
Reagent No. 3, $K_2O$ .....	236
Reagent No. 4, $P_2O_5$ .....	236
Reagent No. 5, N.....	236
Reagent No. 6, N.....	236
Reagent No. 6A, N.....	237
Reagent No. 7, N.....	237
Reagent No. 8, N.....	237
Reagent No. 9, N.....	237
Reagent No. 10, $K_2O$ .....	237
Reagent No. 13, $CaO$ .....	237
Reagent No. 14, $CaO$ .....	237
Reagent No. 15, total N.....	238
Reagent No. 16, total N.....	238
Reagent No. 17, total N.....	238
Reagent No. 18, total N.....	238
Reagent No. 19, $CaO$ .....	238
Reagent No. 40, $P_2O_5$ .....	238
Series 100 phosphate fixation solution.....	238
Series 500 phosphate fixation solution.....	238
Series 1000 phosphate fixation solution.....	238
Stannous chloride solution.....	238
Ammonium molybdate solution.....	239
Bromthymol blue pH indicator.....	239

Bromcresol green pH indicator.....	239
Chlorphenol red pH indicator.....	239
Phenol red pH indicator.....	239
Hydrochloric acid .....	239
Nitric acid .....	239
N/2 hydrochloric acid solution.....	239
Potassium sulfate .....	239
R.C.M. Color Standards.....	239
Soil phosphate .....	240
High-register soil phosphate .....	240
Phosphate in cane juice.....	241
Soil phosphate fixation.....	241
Soil nitrogen .....	242
Detailed R.C.M. Procedures.....	243
Rapid Estimation of Available Nitrogen in Soils.....	243
Rapid Estimation of Total Nitrogen in Filter Cake.....	246
Rapid Estimation of Total Nitrogen in Soil.....	248
Rapid Determination of Nitrogen in Mill Water and in Water Containing Organic Matter .....	249
Rapid Determination of Nitrogen in Irrigation Waters.....	250
Rapid Estimation of Nitrogen in Cane Juice.....	251
Rapid Chemical Methods Nitrogen Studies.....	254
Plan of experiment.....	254
Collection of samples and preparation for analysis.....	256
Analysis of plant material .....	257
Rapid Estimation of Total Nitrogen in Molasses.....	258
Rapid estimation of Phosphate in Soils.....	259
Phosphate Fixation .....	261
Rapid Estimation of Phosphate in Cane Juice.....	263
Rapid Estimation of Phosphoric Acid in Boiler Water.....	265
Rapid Estimation of Phosphoric Acid in Filter Cake.....	265
Rapid Estimation of Potash in Soils.....	267
Rapid Estimation of Potash in Cane Juice.....	271
Rapid Estimation of Potash in Molasses.....	273
Rapid Estimation of Potash in Irrigation Water.....	274
Rapid Estimation of Potash and Phosphoric Acid in Mill Ash.....	278
Rapid Estimation of Calcium in Soils.....	281
Rapid Estimation of Calcium in Filter Cake.....	285
Estimation of Soil Reaction (pH).....	286
Plantation R.C.M. Laboratories .....	289
Bibliography .....	299

#### FOREWORD

The one per cent citric acid soluble method of soil analysis has been supplanted almost entirely on Hawaiian sugar plantations by rapid chemical methods\* of soil and

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\* Popularly referred to in Hawaii and hereafter in this paper as R. C. M.

plant analyses. With few exceptions these analytical determinations are being made by plantation men in plantation agricultural-chemical laboratories. A cooperative enterprise consisting of plantation and Experiment Station workers has come into being, having received its initial impetus as the result of the pioneering efforts of a few plantation men and members of the Extension Service Staff of the University of Hawaii.

It is the purpose of this article to discuss the development of the present-day R. C. M., a portion of which had its foundation in the earlier "kit" ensembles. However, before going into details of this development, a better understanding may be realized of the objectives sought in R. C. M. if a brief review is presented of the general plan of the one per cent citric acid soluble soil analysis project and if a few of the more prominent reasons which led to its decline are cited.

### THE CITRIC ACID SOLUBLE PROJECT

For purposes of discussion the "citric soluble" project may be divided into:

1. Collection of soil samples.
2. The plantation survey.
3. The analyses.
4. The report to the plantation.
5. Interpretation of data.
6. Reasons for its decline.

#### *1. Collection of Soil Samples:*

Two members of the Experiment Station Chemistry Department would visit a plantation, sampling, usually, all the fields upon which cane was growing or which, at that time, were in temporary fallow or in preparation for harvesting, planting, or ratooning. Three plantation laborers would be assigned to assist the visiting chemists. Soil borings were collected, as a rule, from 12 points about 75 feet apart in a field or part of a field. These specimens would be taken from the first foot, and occasionally from the second- or third-foot zones. The 12 borings would then be composited into one sample, about 15 pounds being retained for analysis.

#### *2. The Plantation Survey:*

For purposes of expediency and economy the visiting Station men would endeavor to obtain representative soil samples from every field under cultivation, provided it were possible to penetrate growing fields and get about in them. Depending upon the size of the plantation and upon the ground to be covered, the survey would require 1, 2, 3 days, or longer. Usually other plantations on the island would be surveyed during the trip.

#### *3. The Analyses:*

Upon arrival in Honolulu the soil specimens would be air-dried, disintegrated by pounding, screened and placed in labeled glass containers. Total phosphates and potash were determined as a rule and also potash, phosphate and lime extractable by strong hydrochloric acid. Chemical treatment of the soil for the one per cent citric

acid determinations consisted of a primary overnight maceration of soil and citric acid solution, adjustment being made the following day to a trifle over one per cent free acid in the soil-solution mixture. The extraction was concluded with a continuous mechanical agitation of the mixture for 6 hours. The extracted soil nutrients determined in the analysis were: potash, phosphate, lime, and silica.

Soil reaction (pH) determinations were made immediately following the receipt of the collected specimens in the laboratory.

#### *4. The Report to the Plantation:*

The time elapsing between the collection of soils on the plantations and the mailing of the data and recommendations from Honolulu usually varied between 3 months and 1 year. The duration of this interval was governed by the amount of similar work already in process or being held for attention in its turn and by the number of chemists available to carry on.

#### *5. Interpretation of Citric Soluble and Associated Data:*

The value of "total" and "strong hydrochloric acid soluble" nutrient data cannot be questioned, for they serve a useful purpose. Except in a relative sense, however, total and hydrochloric acid soluble figures have little value in arriving at the amounts of soil nutrients present in an immediately available form. The one per cent citric acid soluble data were considered as representing "available" soil constituents. It has been the custom to recommend specific fertilization when the citric soluble potash figure fell below .04 per cent and the phosphate below .005 per cent.

The employment of "citric soluble" data as a measure of availability has never received the unanimous support of plantation men nor of the majority of research workers in the field of soil science. It is not the purpose of this paper to include discussion bearing upon objections to the citric soluble method of soil nutrient appraisal except insofar as the objections apply to the abandonment of the method in favor of the more rapid analyses which are now in vogue.

#### *6. Reasons for Its Decline:*

With the foregoing brief summary, chiefly of citric soluble technic, we arrive at a consideration of the reasons given by a number of plantation men for not feeling entirely justified in attempting to use citric soluble data in the formulation of a *current* program of field fertilization.

The nature of the survey renders it expedient to include, for the purpose of soil collection, every field on the plantation that it is possible to enter. This situation entails the sampling of areas at various intervals following fertilization and at various stages of cane growth. Instances have been noted in which fields of young cane have been sampled a week or even a few days following the application of mixed fertilizer. Another objection, equally as serious, has been the unavoidable but quite unsatisfactory delay which necessarily ensues before the analytical data are made available to the manager and his staff. A third objection frequently voiced has been inadequate soil sampling, not only regarding the questionable matter of any given sample representing the immediate fertility of the location from which it may

have been collected—we still have that problem—but more strongly against a composite soil specimen selected from a single random field block which is not equal, in some cases, to one twenty-fifth, or at times to one one-hundredth or less of the area it is expected to represent. Here again we find a fault not due to any inherent defect of the citric soluble survey, except wherein limitations of Experiment Station personnel, costs of analyzing the soils in Honolulu, equipment and chemists to handle a great number of soil specimens (comparable to those collected for the plantation rapid analyses) render a more detailed general survey an economic impossibility. Individual 2-acre, or even 5-acre soil composites collected from harvested fields of all plantations could scarcely be analyzed regularly in Honolulu by the old methods with a chemistry staff of approximately 15 analysts. But it should be noted that this accomplishment has been achieved with R. C. M. on several plantations within a harvesting season by one trained analyst and a helper.

#### THE INTRODUCTION OF SOIL-TESTING KITS IN HAWAII

The foregoing discussion is offered as a background to clarify somewhat the dissatisfaction of plantation men which made it evident that a radical change in the method of soil analysis was not only imminent, but urgently needed.

#### RAPID CHEMICAL ANALYSES NOT A NEW IDEA

The examination of soils by rapid methods of analysis for determining concentrations of available plant nutrients is not a recent innovation. In his text on *Soils* published in 1906, E. W. Hilgard (6) includes an appendix by Loughridge, entitled "Short Approximate Methods of Soil Examination Used at the California Experiment Station." In the text of the appendix Loughridge describes procedures for estimating concentrations of phosphates, lime, humus, and alkali salts in soils, any one of which may be completed within one hour. The determination of potash in soil is also described but the analysis required about 3 days for completion.

#### · PROF. F. G. KRAUSS AND THE EXTENSION SERVICE OF THE UNIVERSITY OF HAWAII

In his early association with Prof. Hilgard in California, Prof. Krauss, recently retired Director of Agricultural Extension Service, University of Hawaii, found that useful and practical applications of the rapid methods of soil analysis were being made at the California Experiment Station and that in general they had been found satisfactory in classifying agricultural lands and useful in formulating plans for fertilization. Later, in his agricultural extension activities in Hawaii, Prof. Krauss felt the need of rapid analytical methods for examining soils and in due course conducted experimental studies using several Mainland-manufactured, soil-testing kits. He found limitations in the use of the kits and noted features concerning the technic of the kit determinations which were objectionable. Nevertheless, he definitely established the fact that a marked deficiency of any one of several major plant nutrients could be ascertained by kit soil tests. In several cases his kit findings were verified by pot and field plot tests. "Trends" of nutrient deficiencies, as determined by quick soil tests, were substantiated to a degree by chemical analyses conducted by several colleagues.

## GROVE FARM INVESTIGATES SOIL TESTING KITS

In the autumn of 1931 Prof. Krauss, in a conversation at his office in the University with E. H. W. Broadbent, Manager, Grove Farm Company, Ltd., Kauai, discussed the results he had secured in the studies then under way with the soil-testing kits. He suggested to Mr. Broadbent that the advantages of the rapidly conducted kit tests embraced not only small costs of analysis but that the scheme rendered it possible to examine a large number of individual soil specimens from comparatively small areas. Later, by arrangement with Prof. Krauss, Mr. Broadbent was given, at Lihue, a demonstration of the Urbana kit test (1) for soil phosphate by J. C. Thompson, County Agricultural Extension Agent, University of Hawaii. Following this demonstration, Mr. Broadbent ordered one of the phosphate soil-testing kits and one Urbana potash kit which had just been placed on the market, delivery being made at Lihue in December of 1931. W. P. Alexander, Assistant Manager, Grove Farm Company, Ltd., thereafter conducted, we believe, the first experimental tests of these kits in Hawaii upon sugar cane field soils. In January of 1932 Mr. Alexander established definite correlation between the one per cent citric soluble soil data for potash and phosphate and the groupings of nutrient concentrations found in his studies by the kit tests. He found a marked similarity in trends in comparing the two methods of analysis when the results of either were tabulated by grouping the data in designations of low, doubtful, medium and high.

## THE EXPERIMENT STATION INVESTIGATES KIT PROCEDURES IN THE FIELD

Mr. Alexander continued his studies during 1932 and in June of that year turned over the general investigational details to A. Ayres, assistant chemist, Experiment Station, H.S.P.A., the latter having gone to Kauai to devote his attention to this work.

Mr. Ayres established a laboratory in a building which was very generously placed at the disposal of the Experiment Station by the Lihue Plantation Company, Ltd. He received the cooperation and assistance of A. M. McKeever, B. B. Henderson and other staff members of the plantation in equipping the laboratory. Mr. Ayres proceeded to study field applications of kit analyses, the object being to determine the limitations of the kits and the possibilities of their employment in soil nutrient survey studies. The laboratory was intended originally as headquarters for conducting field studies.

## THE FIRST DEPARTURE FROM CONVENTIONAL KIT TECHNIC

In a short space of time Mr. Ayres found it difficult to perform consistently, with any degree of accuracy, certain portions of the prescribed kit soil analyses without introducing numerous modifications which were suggested, of course, by his previous training and experience. His tests ceased to be confined to the equipment of the "laboratory in a tin box." He added several standard laboratory devices to the technic of work and noted hindrances which developed in the prosecution of the tests. He found also that phosphate could not be determined by the kit method on certain Kauai soils, due to the highly colored nature of the extracting solution following contact with the soil. He experienced difficulty in making the potash tests

because of the unsatisfactory method of manually controlled rotary mixing of soil extracts and reagents and because no constant source of artificial light was provided, calibrated in an optical device, to permit reasonably accurate turbidimetric readings in the final potash test solution. These and other improvements were to appear later by other workers but Mr. Ayres was forced to improvise an optical instrument for making test readings in the soil phosphate determination and to filter off the soil extract from the soil specimen preparatory to developing the critical color reaction of the test. Difficulty of a chemical nature developed when he attempted to make phosphate tests on calcareous soils. The extracting solution furnished with the kit is mildly acidified with hydrochloric acid. In the presence of calcium carbonate of the coral this free acidity may be partially or entirely neutralized, the result being a faulty extraction and failure to obtain in solution the available phosphate contained in the soil specimen. This difficulty was also overcome later at the Experiment Station in Honolulu.

#### EXPERIMENTAL RESULTS OF FIELD TRIALS USING SLIGHTLY MODIFIED KITS

Having determined the limitations of the kit tests and having introduced modifications in the technic of the soil phosphate determination, Mr. Ayres proceeded with the field kit study on Kauai soils with a degree of progress quite satisfactory under the limitations of the study. He found, for instance, that it was a feasible undertaking to sample and analyze a very large number of soil specimens from a single field and to seek in the resulting data information relative to the uniformity of the field with respect to the nutrient in question. In this manner he established the fact that a wide degree of variation existed in the concentrations of available phosphate in the average cane field of Kauai. In cooperation with J. N. P. Webster, then Island Representative of the Experiment Station on Kauai, Mr. Ayres worked up correlations between field experiment and kit data on available soil phosphate. As a result of this work the conclusion was reached by these men that the response of sugar cane to phosphate fertilization on Kauai soils should not be expected if the supply of this nutrient in the field exceeded an amount corresponding to that determined as "doubtful" on the kit scale.

At this time Mr. Ayres was also able to show by the comparison of kit analyses of field soils with adjacent virgin areas that in the case of acid soils practically all of the phosphate extracted in the kit analysis came from added fertilizer and that virgin acid soils, even though containing some available phosphate, failed utterly to give indications of the fact by regulation kit analyses. This finding indicates that naturally occurring phosphates in certain acid soil types are totally insoluble in the kit extracting medium.

#### A LABORATORY STUDY OF THE ORIGINAL KITS IN HONOLULU

Somewhat prior to Mr. Ayres' work on Kauai, L. E. Davis gave attention to the study of a number of popular soil-testing kits in the chemistry laboratory of the Experiment Station in Honolulu. A final step in the soil phosphate determination of one widely used kit involved the stirring with a tin rod of a supernatant liquid in a test vial above a quiescent settling of the soil specimen under examination. This

operation developed a blue coloration in the test liquid directly proportional in intensity to the amount of phosphate extracted from the soil below it.

Mr. Davis found that, with many Hawaiian soils, this supernatant liquid layer, lying above the soil in the vial, was frequently highly colored with organic matter, iron compounds, etc., and quite often was extremely turbid because of the presence of a finely divided suspension of soil which would not settle for hours or in some instances for days. In cases such as these it was futile to attempt an estimation of the blue color in the orange colored turbid or muddy liquid medium for it is upon the intensity of the blue color developed in this medium that the analyst estimates his quantitative reading. The blue coloration produced with the tin rod is due to the reaction of the tin with compounds of molybdenum and phosphorus. Under properly standardized conditions the intensity of the color thus developed becomes a very accurate measure of the quantity of phosphate present. One of these conditions requires a narrowly defined range of acidity. If the acidity becomes too low, deep blue compounds of molybdenum are formed which contain no phosphate whatever. Obviously, in this circumstance, the color of the solution is of no value in estimating the phosphate extracted from the soil. On the other hand, if the solution is too acid the blue color is not formed even when large amounts of phosphate are present.

Hence, Mr. Davis encountered difficulty as did Mr. Ayres in working with alkaline or coral soils which partially or entirely neutralized the feeble acidity of the kit reagent used in the extraction of the soil. With these difficulties in mind, Mr. Davis attempted to adapt the kits to as wide a use as possible. He too found it essential to depart from the "laboratory in a tin box." Improvement came immediately in the modification of the procedure whereby the extracted soil was removed from the extracting reagent by filtration and where the critical blue color was developed by using a solution of stannous (tin) chloride in place of the tin rod. The test was still restricted, however, to non-calcareous soils. A second improvement, devised by Mr. Davis, with the cooperation of E. K. Hamamura, consisted of an extraction of the soil with dilute hydrochloric acid in place of the kit reagent. The filtered extract was then evaporated almost to dryness and the residue was dissolved in the proper reagent. This change made the method applicable to calcareous as well as non-calcareous soils.

#### DIFFICULTIES WITH THE POTASH SOIL KIT

In his study of a commercial kit method for the estimation of available potash in soil, Mr. Davis found that the composition of the potash compound producing the turbidity in the test—the important last step in the determination—varied considerably, not only with the amount of potash extracted from the soil, but, unfortunately, with the manner of adding reagents to the soil extract and in the character of the manipulations used by the analyst in the analysis. In this test the estimation of potash involves the formation of sodium-dipotassium-cobaltinitrite in the test solution. This compound is produced by the analytical procedure, generally, in very finely divided form so that the mixture becomes turbid. When properly conducted the turbidity so produced is related directly to the amount of potash



extracted from the soil. The difficulties encountered with the potash kit were overcome later and will be described in more detail.

#### EXTENDED EXPERIMENTAL TRIAL OF THE KITS ON A FEW PLANTATIONS

Interest in the kit methods of soil analysis, while not widespread among Hawaiian sugar plantations at this time (1932), was nevertheless on the increase. But, while interest grew in the kit experiment, dissatisfaction also developed because of the irrational results obtained on our many prevailing soil types and because of the failure of those investigating the methods to secure consistent analytical checks.

It must be emphasized that the principal commercial soil-testing kits were extremely simplified assemblies of apparatus and chemical reagents intended primarily for the individual farmer who may have had little or no experience whatever in using equipment of the kind. Much less would he be likely to devote enough of his time and attention to the study in order to interpret his data in a manner which would include correlations with replicated field experiment and other exacting procedures of scientific soil evaluation.

#### SOME ADVANTAGES OF ORIGINAL KIT ASSEMBLIES

Before proceeding further in pointing out what we believe to be disadvantages of kit methods in general, it should be emphasized that of all the commercial kits investigated at the Experiment Station in Honolulu, with one exception, it was gratifying to discover that they were not designed to mislead anyone and that they were sound in their fundamental principles. It is also true that had it not been for the simplicity of the kit tests and for the rapidity with which an analysis could be made with them—unreliable as they proved to be with some Hawaiian soils—it is indeed doubtful if the intensive experimental study of rapid chemical methods would have received the stimulus it did among plantation workers.

With one of these kits a farmer could enter his fields, procure a sample of soil and immediately analyze it on the spot. This distinctive “kit” feature led to the undeserved quasi-derisive sobriquet for the kits of “running board laboratories.”

#### OTHER OBJECTIONABLE FEATURES OF COMMERCIAL KITS

The experiences of plantation investigators and the chemistry staff of the Experiment Station soon brought to light the fact that the limitations of the soil-testing kits were many and frequently were serious. They were inaccurate not merely in the sense that results could not be expressed to as high a degree of precision as those obtained from the prevailing analyses of citric acid soil extracts, but in the more important sense that they were in many cases utterly unreliable.

#### A PLANTATION STAFF CITES ITS KIT EXPERIENCES

Apropos of the difficulties encountered by plantation investigators we quote in part a letter received by the author in May 1936 from Wm. Campsie, Manager, Hutchinson Sugar Plantation Company, Naalehu, Hawaii. He wrote: “I can remember very well our first experience with those kits. The phosphate kit was good

in certain kinds of soil, but I was tempted many times to scrap the potash kit as we could get no correlations with juice sampling or Mitscherlich tests and could not even duplicate our results on the same soil from day to day.

"Only Hossack's tenacity and perseverance kept us going with it at that time." (The time referred to by Mr. Campsie was in the summer of 1933.)

Mr. Campsie's reference to Mr. Hossack's tenacity and perseverance in staying with the kits is particularly appropriate in its bearing upon the development of R.C.M. as it exists today in Hawaii. One by one, plantation workers were showing unmistakable lack of interest in kit studies. Investigational kit study at the Experiment Station (already referred to) was of necessity slow in bringing about proven modifications which could be recommended for general adoption. Mr. Hossack, however, hung on and in the face of waning Hawaiian interest in the kits his perseverance bridged a gap which otherwise might have brought about the termination of plantation interest in any form of rapid soil analysis.

The author requested Mr. Hossack to recount his experiences in the evolution of the kit tests to the modern rapid chemical methods. He has very kindly contributed an interesting and concise account of his work with Mr. Campsie which is quoted directly below.

The original Urbana soil-testing kits were first used at Hutchinson Plantation about the end of 1932, and by the beginning of 1933 a regular system of soil sampling was inaugurated. A small amount of experimentation was necessary in order to decide on the best method of conducting a soil survey of the whole plantation, but very little time was lost, and an intensive system of soil testing was well under way in January of 1933, and our records of this work are complete from that date up until the present time.

The system that was adopted was to collect a representative number of soil samples from each field immediately after harvest. In the early work 1 composite sample from 5 to 8 acres was the general rule, but as the value of this work was recognized more time was set aside, and with the help of one laboratory assistant more and more samples were collected, so that by the beginning of 1934 it was possible to sample all fields on a 2-acre basis.

A regular system of correlating kit analysis with citric soluble analysis and results from Mitscherlich tests was also started right at the beginning of the soil survey. During the early period it was sometimes difficult to get a correlation between the various methods. Many difficulties were encountered, errors in Mitscherlich tests, faulty reagents and imperfect apparatus, all caused a great deal of inconvenience, but where all factors showed a definite correlation, we were able to conduct a fairly elaborate system of spot fertilization. When there was any doubt we erred on the safe side by applying more fertilizer than was necessary.

There is no doubt whatever but that the gradual perfecting of the kits by the chemistry staff of the Experiment Station did more than anything else to establish confidence in the data secured from the soil survey. Both the phosphate and potash kits were far from being reliable in their original form.

The high organic nature of some of the soils of our upper fields caused a very erroneous estimation of their phosphate content, and it was not until the perfected "Laboratory Method" was introduced that we were able to secure accurate data. Fortunately our lower fields were low in organic matter, and the original kit analysis showed us a fairly accurate picture of our phosphate supply in these areas. In this manner we were able to omit phosphate on a considerable area where all methods of analysis showed a high amount. We did little spot fertilization with potash in the very early days, and it was not until the advent of the Potash Illuminator and later of the Potash Rotator that we were able to place a high degree of confidence in our potash analyses.

The whole history of the development of a systematic soil sampling program on this plantation has been one of continuous improvement. New methods of technic, discarding of

faulty and cumbersome systems, confidence in the data secured, have all helped to establish a system of fertilization control that is saving the plantation thousands of dollars a year and has given us a newer and clearer understanding of our plant food requirements.

### THE HAWAIIAN SOIL PHOSPHATE FIXATION KIT

The former Director of the Experiment Station, H. P. Agee, contributed his support and interest to an exhaustive study by the writer and other chemistry department members of kit technic with Hawaiian soils in an attempt to modify them sufficiently to meet sugar plantation requirements or to develop new or original rapid methods of analysis to meet Hawaiian soil conditions.

One of the first developments in this venture was the appearance of the Experiment Station, H.S.P.A. soil phosphate fixation kit. This rapid methods assembly was developed by the director, the writer and Q. H. Yuen of the Chemistry Department. Its use enabled the cane grower to ascertain the degree of "tie-up" or fixation of phosphate which may be expected in his fields following the application of a soluble phosphate fertilizer. This assembly was later provided with an adjunct which permitted additional estimations to be made of intensity and rate of soil phosphate fixation. This assembly, its development and uses, has been previously described (3). Additional comments will not be made here except, perhaps, to state that the fixation assembly is in wide use today, with no new modifications having been necessary since the addition of the adjunct feature in the fall of 1934.

### DEVELOPMENTS REGARDING DEPARTURES FROM COMMERCIAL KIT PROCEDURES

Through a great number of disappointing setbacks, the kit features of the various soil tests gradually gave way to more precise means of estimating the concentrations of available soil nutrients. Procedures were developed, in some cases, where radical modifications were made from the technic of the original kits. New equipment and severe simplicity and straightforwardness of analytical detail were maintained; so likewise was the important feature of rapidity of analysis. As they accrued and were found useful and practical, improvements and newer developments were submitted to the plantation users for criticism and cooperative study. One of the features of the kits first abandoned was the use of the opaque, printed color chart to compare developed test colors with standard printed variations of a definite shade. In its place was substituted clear solutions of organic dyes sealed in vials which were duplicates of the test vials used in the analysis.

Rapid chemical soil analyses would be difficult of accomplishment were the chemist to be deprived of the use of color phenomena as indicators of completed reactions or as measures of concentrations of various soil constituents which lend themselves to quantitative color reactions.

Laboratory analyses in R.C.M. work are, as a rule, conducted in such a manner that the final test solutions are placed in thin glass vials preparatory to the development of the distinct shade of color in clear solution, opacity or turbidity which is directly comparable with the concentration of the nutrient which is sought in the analyses. When "shade of color in clear solution" is the measure of the nutrient sought, comparison of the test with opaque, printed charts (in the ordinary variable sources of light in the laboratory) is far from being a satisfactory or reliable pro-

cedure. For this reason we adopted the long-established system of making color comparisons by the use of a standard source of artificial illumination. The unique features of the departure from kit, printed color charts embraced the selection of vials identical with those used in the various tests for carrying standard colored solutions for comparison purposes, the development of illuminating apparatus, racks in which to make the comparison, etc., and of the standard colored solutions themselves.

#### THE GRADUAL EVOLUTION FROM KIT TESTS TO R.C.M.

With the beginning of studies in the preparation of satisfactory color standards Messrs. Yuen, Hamamura, Nishimura and Chu of the chemistry staff were assigned to various research topics leading to improved technic in analytical procedure. Researches continued as progress was made, these same investigators devoting their entire time to assisting in the development of new chemical methods of analysis and further refinements in established procedures.

Many outstanding chemical and mechanical developments in the present R.C.M. complement of assemblies have been due entirely, originally or individually to the efforts of Messrs. Yuen, Hamamura, Nishimura and Chu.

Dr. H. L. Lyon, Director of this Experiment Station, members of the chemistry and agricultural staffs, the plantation staffs and the author have contributed other details.

To avoid countless repetition and frequent acknowledgment of individual effort in the following description of R.C.M. developments, no attempt will be made to associate in any more detail the growth of the enterprise with any of the personnel who have contributed to the work.

#### COLOR STANDARDS

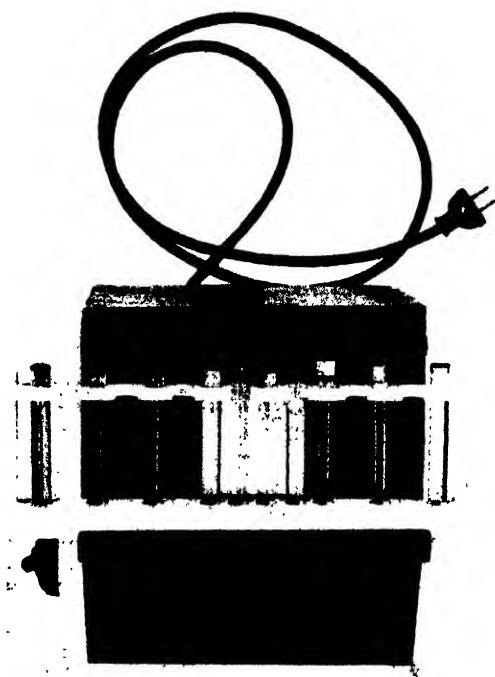
Attempts to prepare durable and permanent liquid color standards were fraught with difficulties. Aniline dye solutions which were fast to light in quartz or "non-sol" glass containers slowly deteriorated when put in service over artificial light in the regulation test vials closed by rubber stoppers. Marked improvements have been obtained in maintaining the permanence of these standards and in extending their useful life by impregnating the container stoppers with a nitro-cellulose compound and in peptising the dye solutions (which are colloidal) with appropriate stabilizers.

Satisfactory standards of an improved nature have been prepared from solutions of inorganic salts wherever the colors obtained from such sources may be used. Research at present is devoted to the substitution of stabilized inorganic permanent standards for the naturally fugitive dye solutions, the latter having been found unable to withstand prolonged usage without gradual deterioration. At the time of this writing (August 1936) the useful life of the most fugitive of the R.C.M. color standards has been extended to four or more months.

#### COLOR COMPARISON AND TURBIDITY APPARATUS

The adoption of liquid color standards for comparison purposes in rapid colorimetric analyses necessitated the designing and building of suitable apparatus to conduct the tests. For the determination of phosphate a ventilated wooden box was

designed to enclose a calibrated electric light bulb which could be turned off and on by a switch placed at a convenient point outside. The light within the apparatus was released through a dense, opal glass window built in a plane  $45^\circ$  from the horizontal. By means of a permanently attached recessed guide placed on the outer surface of the device and under the illuminated window, a notched rack can be accommodated. The rack can be made to slide back and forth in front of the window. The sliding rack is provided with circular openings between the notches, the former carrying the color standard tubes in graded shades of increasing intensity from left to right. The notched indentations in the rack were gauged to permit the ready



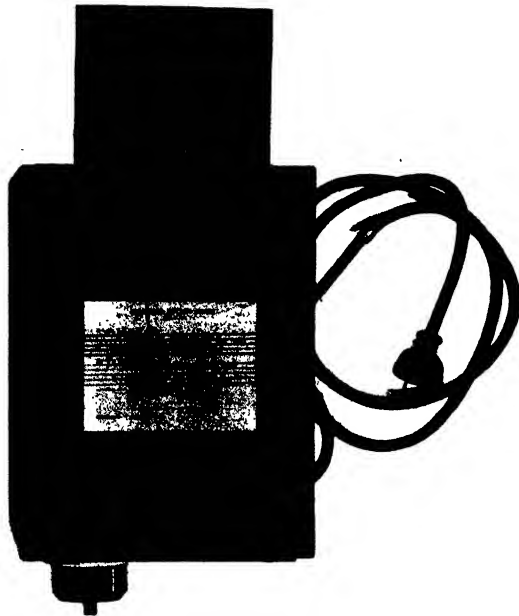
The H.S.P.A. Phosphate Illuminator.

insertion and removal of the analytical test vial from point-to-point along the rack. By this means the shade of color of the solution under examination can be matched with the appropriate standard in a field of constant, diffused artificial light.

In the potash determination the end point or critical period of the analysis depends upon the measurement of turbidity produced in the test by a finely divided orange-colored precipitate. The Urbana Laboratories (2), the originators of the soil potash kit using this principle, employ a chart in separate gradations of fine, medium and heavily ruled series of lines as a means of gauging the turbidity produced in this test. The vial containing the turbid orange-colored suspension is held by the analyst about one-half inch above the ruled chart and, while sighting down through the test liquid, the vial is moved across the separately spaced groups of ruled lines until any one of the series of lines may be distinguished through the tur-

bid liquid. The ability to just distinguish any one of the lined groups constitutes the final step in the analysis. (More details will appear later under the description of the R.C.M. determination of potash.)

There are two serious objections to this procedure, both as to convenience and consistent accuracy of analyses. Uncertain source of natural light is one objection and difficulty of holding test vials always exactly one-half inch from the chart is another. To overcome these faults the potash illuminator was developed. This device is similar to the box-like phosphate illuminator containing a calibrated electric light bulb and ground glass window. In the potash illuminator the window of the instrument is placed on the horizontal upper surface of the device. In a recessed opening directly above the window is placed a printed lined grid, covered by a piece of clear glass which is just flush with the upper surface of the instrument. A slide, perforated to accommodate 3 test vials, may be moved back and forth in the field of light between 2 lateral guides permanently attached at the edges of the upper surface. By this means turbidity may be measured by sighting through the column of test liquid upon a uniformly lighted field which carries the ruled grid or chart. The 2 illuminating devices just described appeared in the spring of 1934.



The H.S.P.A. Potash Illuminator.

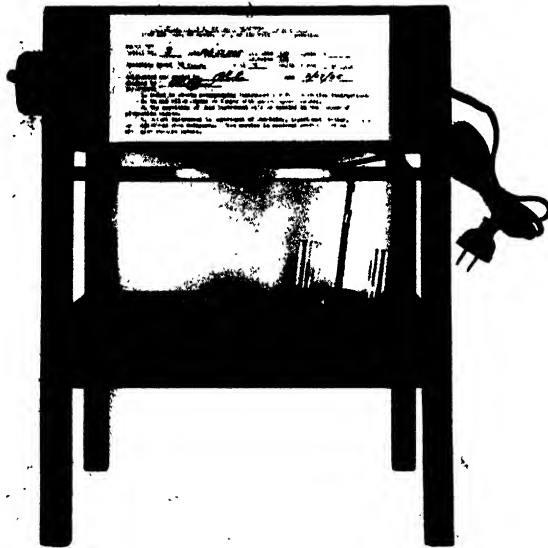
#### THE POTASH ROTATOR

Up to September 1934 some progress had been made in the establishment of individual agricultural soil-testing laboratories among the 39 plantations of the

Association. Progress was slow, however, chiefly because of the difficulties encountered by plantation men in securing consistently accurate checks of analyses of soils for readily soluble potash and phosphate. (Potash, phosphate and soil reaction were the only analyses made at this time.)

The introduction of the Potash Rotator was followed by a marked stimulus of plantation interest in conducting plantation soil studies. This instrument also greatly improved the accuracy and reliability of the determination of potash.

In the early potash kit procedure, the analyst was instructed to agitate 4 drops of a reagent with 1 ml. of soil extract in a short glass vial provided for the purpose. This step was followed by the introduction of 1 ml. of a second reagent so that the latter would form a clear distinct upper layer upon the solutions previously placed in the vial. Up to this point no difficulties were encountered by anyone after having practiced the various steps involved. However, the next operation requires an un-



The H.S.P.A. Potash Rotator (side view).

usual degree of skill if consistent results are to be regularly obtained. The analyst takes the vial in his hand and for a period of one-half minute he agitates the contents in a gentle circulatory manner so as to produce a distinct type of crystalline turbidity which is the measure of the potash occurring in his "unknown" soil extract. It was this feature of the potash determination which appeared to be responsible for the widely variable results turned out by even the most experienced analysts doing this work. Our efforts at the Experiment Station in Honolulu were

directed towards devising a mechanical instrument which would always perform this difficult operation in the same manner and without variation or change when once correctly adjusted.

In August of 1934 the first Potash Rotator was built which met all the requirements imposed by a very exacting manipulation. Improvements upon the original machine were made from time to time. The present Potash Rotator consists of a



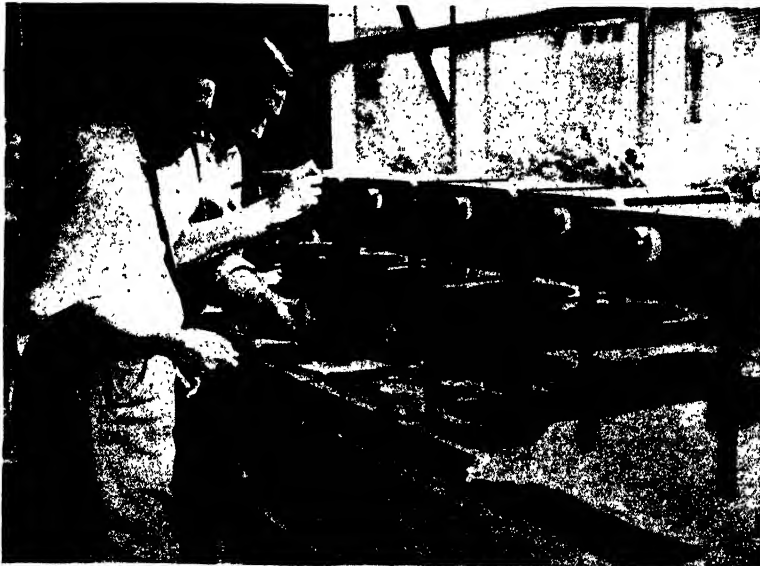
The H.S.P.A. Potash Rotator (portable). Designed for plantation demonstrations.



double-decked frame about 28 cm. square and 36 cm. high. The upper deck supports and encloses an induction-type electrically driven motor and mechanism which has been adjusted to turn a 29 cm.-flywheel in a horizontal plane directly beneath the upper deck. At a distance of 8 cm. from the center of the flywheel, on its lower side, a 3 mm. round brass shaft, 15 cm. in length, is attached by a hinged joint so



The author and W. F. Burton, machinist, in the Experiment Station, H.S.P.A. shop.



H.S.P.A. Potash Rotators being manufactured for shipment to plantation R.C.M. laboratories.

that it hangs, normally, in a vertical position (at right angles to the plane of the flywheel) and tends to be thrown out from the center of the flywheel by centrifugal force when the wheel is turning. The brass shaft supports a disc-like, rubber-covered wooden rotor, the latter 8 cm. in diameter and 4 cm. in height and free to rotate about the shaft. The rotor is provided with 4 equally spaced bored receptacles, 3 cm. deep, to carry snugly 4 of the test vials used in the potash determination. When in operation the rotor travels within an elliptical wooden guide, the latter parallel with the plane of the flywheel and situated 11 cm. below it. The inner sweep of the elliptical guide opening measures 22 cm. short diameter and 25 cm. long diameter. It forms the upper bracing of the lower deck, the machine being supported below it by 4 corner legs. In operation the rotor turns in a direction counter to the flywheel, its orbit following the elliptical guide, contact with which causes its rotation about the shaft. The net effect is a distinct oscillatory, rocking motion impressed on the liquid contents of the test vials which simulates the desired rotary stirring action as conducted by an expert in this exacting operation. The flywheel is driven at 78 r.p.m. and the duration of rotation is fixed at one-half minute.

The Potash Rotator has been found entirely satisfactory and during the 2 years of its existence, 115 instruments have been manufactured and put in service. Following the rotation of the test solutions, the vials are transferred to the Potash Illuminator for the final reading, details of which will appear later.

#### THE DETERMINATION OF PHOSPHATE IN SOIL

Following the introduction of the Potash Rotator attention was directed to improving the technic of the soil phosphate determination. The objections which still obtained, even in the modified kit procedure for the determination of soil phosphate were in some degree due to the peculiarities of the average Hawaiian soils. Organic matter, and in some cases appreciable amounts of coral brought into solution in the extraction of the soil, resulted in the development of such high discoloration or chemical distortion in the extracted soil solution that the original or modified kit method could be used with success only with soil types which did not contain objectionable constituents.

An attempt was made, therefore, to develop an entirely new rapid analytical procedure which could be employed with assurances of reliability and accuracy in all prevailing Hawaiian soil types. The final conclusion of this research gave us an entirely satisfactory and foolproof method. A detailed description of the method will be found later in this paper. The objections to the kit procedure were overcome by extracting the soil specimen with a new, weakly acidified reagent, destroying dissolved organic matter by nitric acid, converting carbonates and bicarbonates to non-interfering chlorides and redissolving the treated soil extract residue with appropriate reagents, thereafter proceeding with the analysis. The unavoidable solution of iron compounds from a few soils results in the formation of a pale yellow coloration which is carried through all the stages of the analysis. When this condition is met with, the persistent yellow tinge in the test solution blends with the blue color developed in the test and turns the normally pure blue to a shade of

green. As a compensating expedient we introduced additional series of blue-green color standards which have been found quite satisfactory in meeting the difficulty.

#### A CENTRAL DEPOT FOR REAGENTS AND APPARATUS

The improvements to the potash and phosphate determinations were reflected in a greatly increased expansion of plantation study of soil matters.

In December of 1934 the author recommended that all users of the rapid methods agree to employ reagents and special apparatus originating in a single source of supply. This suggestion met with plantation and Experiment Station approval. A building on the Experiment Station premises was fitted up as an R.C.M. annex. Here reagents are manufactured, aged, analyzed, tested, packaged, and stocked for distribution to all plantation laboratories of the Association. Apparatus, accessories, and special equipment used in R.C.M. work are also stocked in this building and shipped as they may be required.

The advantages of this arrangement were manifested from the very first. All members of the Association would employ reagents of uniform composition and quality. The costs of preparation were markedly reduced by handling large volumes of chemicals and supplies. Several important reagents require special care in preparation, using somewhat complicated formulae. Others remain stable only when stocked in paraffined glass containers, while still others slowly decompose after preparation and must be exchanged or renewed at 4-month intervals.

A very important factor related to the central depot arrangement for reagents embraced the matter of the checking, by Experiment Station men, of duplicate specimens of key soils which had previously been analyzed on the plantations. This checking system will be discussed later, it being mentioned here merely to illustrate the advantages accruing to the general Hawaiian R.C.M. enterprise in having a uniform and standard complement of reagents from which all workers secure their supplies.

#### CLASSES OF INSTRUCTION AT THE EXPERIMENT STATION FOR PLANTATION ANALYSTS

As increased numbers of plantation personnel took up R.C.M. studies, a need arose for instituting courses of instruction in the work with practical laboratory analyses included as a part of the training in the technic of R.C.M. analyses. In November of 1933 the first group of 30 men attended classes held at the Experiment Station in Honolulu. The staff of the chemistry department, cooperating, Messrs. Yuen, Hamamura, Nishimura and Chu contributed their services to the attending class which was divided into small groups. In this manner it became possible to offer individual and personal instruction and assistance to each visiting student.

The centralized instruction idea met with immediate response. Classes were held thereafter at frequent intervals. They have been continued at irregular periods as the need has arisen. At present special classes are conducted for the instruction of experienced analysts in newer developments of R.C.M., and regular sessions offering general instruction are continued at occasional intervals.

# SUPPLEMENTING R.C.M. STUDIES OF SOIL POTASH AND PHOSPHATE WITH NEW ANALYTICAL ASSEMBLIES DESIGNED TO EXPAND THE FIELD OF PLANTATION RESEARCH

Satisfactory progress having been made in correlating R.C.M. data with similar findings obtained by independent means gave impetus to the extension of R.C.M. analyses to include the determination of all of the more common nutrients not only in soils but in the cane plant, in crusher juice, in mill by-products, and in irrigation waters. Simultaneously with the development of new analytical assemblies improvements, modifications and additions were made to the older ones. For instance, in the determination of soil phosphate the procedure was adjusted and an additional complement of color standards was prepared which enabled the plantation worker to ascertain with a good degree of accuracy the amounts of available phosphate in soils in which reserves of this nutrient greatly exceeded the ranges usually found. As a matter of fact, reliable information as to the quantities of phosphate in some lands, particularly very large excesses of this nutrient, was not definitely mapped or recorded until after plantation agriculturists had completed their first surveys.

Encouraging progress in correlations suggested a resumption of crusher juice analyses for potash and phosphate. Accordingly R.C.M. assemblies were perfected which enabled an analyst to determine, with more than average laboratory accuracy, the potash and phosphate in samples of crusher juice in less than 10 minutes for both determinations. As nitrogen studies were adopted in the soil work a very satisfactory method was developed for the determination of total nitrogen in crusher juice. To augment the employment of R.C.M. data on plantations, directions for procedure and accompanying data sheets were revised to include percentage and pounds per acre-foot data for determined soil nutrients.

## PLANTATION AND EXPERIMENT STATION CHECK ON ANALYTICAL TECHNIC

Within a period of less than two years a large majority of Hawaiian plantations had adopted R.C.M. studies, had built agricultural-chemical laboratories and had placed the routine detail of analysis in the hands of operators who had been trained at the Experiment Station in Honolulu. As a rule the responsibility of the work, the correlation of data, and field application of findings were in the hands of the plantation agriculturist.

It was apparent that the value of the study and the reliability of the findings depended entirely upon the representative value of the soils and other specimens selected for analysis and upon the accuracy, honesty, and proficiency of the operator conducting the routine analyses. The matter of correct soil sampling and adequate field survey—the vital prerequisites to any analytical study of agriculture—embraces a field of research which is receiving the attention of all those engaged in R.C.M. work. In this paper no further comment will be presented on the details of sampling research, now engaging our attention, because the purpose of the article is essentially an attempted exposition of the R.C.M. fabric. However, the reliability of the work of the plantation analyst is a matter the importance of which cannot be over emphasized. There are several factors which may contribute to faulty chemical analyses. Among these may be mentioned the condition and quality of reagents, inadequate laboratory facilities and improperly adjusted analytical aids,

such as apparatus, color standards, etc. The manner in which these hindrances to analytical proficiency have been controlled will be discussed elsewhere under "Co-operative Features of the Enterprise."

Under the above heading the attention of the reader is directed to a consideration of the important matter of the technic of the R.C.M. operator. Technic of analysis, whether it be good, indifferent or bad, is in the control only of the analyst himself. Any analyst, no matter how expert he may become, is prone to depart from conventional procedures in performing tedious routine and to inadvertently introduce personal modifications in his work. Although they may be slight, nevertheless, errors may develop which may in time become progressively serious, especially where correlations are attempted on a wide scale and interpretation of data obtained by different workers is contemplated. Other operators may become careless, listless or indifferent. Although exceedingly rare, still others may "presume," or to put it mildly, readjust or anticipate a given set of data. A great number of causes or reasons may be responsible for an analyst's tendency to anticipate data. It is equally true that anyone performing routine work, any portion of which will be subjected to analytical check by an independent operator, will give his work the very best he has in him, providing it is his desire to continue on the job.

The author, having discussed this subject with plantation managers and agriculturists and with the analysts themselves, and having received their endorsement, proposed the introduction of a cooperative checking scheme which was adopted in the early part of 1934. The plan of the checking arrangement was quite simple: the plantation analyst would set aside duplicate soil or other specimens which he had analyzed and forward them to the Experiment Station with his data; his results would then be checked with those obtained by Experiment Station analysts and the two sets of data would be remailed to the plantation with constructive comments or recommendations. The value of this feature of the R.C.M. work has justified the pains taken by the analysts to have their data independently checked. When major discrepancies are found the portions of the plantation specimens analyzed at the Experiment Station are returned to the plantation for reanalysis by him. If the second analysis on the plantation does not reveal the cause of the discrepancy then someone from the Experiment Station visits the plantation laboratory and rechecks the questioned data with the analyst in his own laboratory. The visiting Experiment Station worker remains with the plantation analyst until the causes of the discrepancy are ascertained and corrected. This checking system is now an active part of the plantation-Experiment Station cooperation, and it has proved quite beneficial to both participants.

At the present time (August 1936) we have under consideration the recommendation that beginning in 1937 all checking analyses shall be made in the plantation laboratories with the R.C.M. analyst by Experiment Station technicians. This plan, if approved, would terminate the one now in force whereby analyzed specimens are sent to the Experiment Station in Honolulu for checking analyses. It is believed that the advantages of such a modified checking system would be many. The greatest gain, we believe, which should develop from the change would be an immediate straightening out of analytical or other difficulty encountered by the plantation analyst coincident with a failure on his or our part to check his previous

work. Under the present checking system, proficient as it is, such a personal and satisfactory cooperative check is obviously out of the question.

#### COOPERATIVE FEATURES OF THE R.C.M. ENTERPRISE

The inauguration of the checking system for plantation analysts led to the development of an organized cooperative plan of periodic plantation visits by an Experiment Station chemist accompanied by an R.C.M. technical expert. In the early experiences gained in visiting plantations in connection with checking the technic of the analysts, it was found that occasionally reagents had deteriorated by exposure to direct sunlight or from other causes. Perhaps color standards had faded slightly and the fact would not have been realized by the operator. Apparatus may have gotten out of adjustment and hence required realignment or other repair. On the other hand, improvements may have occurred in the plantation manner of pursuing research, for example, in placing soil-sampling stations in the field, in collecting and preparing specimens for analysis and, not uncommonly, in a marked improvement in analytical technic. Faulty reagents or apparatus, of course, required and received immediate attention at the time the plantation visit was made. But equally as important to the enterprise as a whole was the assembling and dissemination to the other plantation and Experiment Station workers of the advancements in the work which accrued as a result of research studies by distantly situated plantation men. An advantage was to be gained, we believed, if one or more persons would circulate among, and keep in closer contact with, the plantation staffs and at the same time attend to the checking system of plantation analysts and offer periodic servicing of all R.C.M. equipment, reagents, and color standards in the laboratories conducting this work. These ideas developed later into tangible form, as will be shown.

Cooperative endeavor at the Experiment Station on R.C.M. researches embraced studies under way in almost all departments. The Director encouraged this move. Chemistry, agriculture, Mitscherlich division of agriculture, pathology, genetics, and sugar technology departments participated in the work. The Island Representatives of the departments of agriculture and genetics actively participated not only in R.C.M. studies on Experiment Station projects, but extended their activities to plantation cooperation.

With the approval of the executives of the Association and the plantation managements, a visiting schedule was organized in the fall of 1934 whereby an Experiment Station chemist would call regularly at each plantation at intervals of 4 months, or more frequently. The chemist was provided with an automobile stocked with a complete complement of all R.C.M. equipment and supplies. Color standards, extra supplies of reagents, special testing apparatus, and kindred sundries were included in the car in amounts sufficient to replace any defective apparatus, reagents or other essentials which might be required by all laboratories engaged in the work. The automobile was shipped on the same steamer which carried the visiting chemist on his mission.

One day or longer, usually one day, would be utilized by the visitor in discussing the work with the plantation manager or agriculturist. This feature of the visit enhanced the contacts between the plantation and the Experiment Station in addition to bringing out subjects for discussion relevant to R.C.M. and other matters

of material interest. Before the day's call was concluded the laboratory operators would be visited and their analytical technic would be checked if they so desired. Repairs or adjustments to special apparatus were made when necessary, all color standards were renewed and replacements made of any reagents which might have become contaminated, overexposed or have shown other evidences of deterioration.

As the plantation laboratories grew in numbers and as the analytical staffs were augmented to carry on additional studies using newly developed R.C.M. assemblies, it became difficult for a single visitor to accomplish all the laboratory detail in a one-day call and also engage the plantation staff in discussions on the progress of work. Then, too, details pertaining to correlations of data were demanding more and more attention as plantation surveys were completed and newer ones inaugurated. To allow the visiting chemist more time to discuss R.C.M. matters with the plantation staffs, it was arranged for a technical expert to accompany the chemist. The laboratory detail on the plantation was then taken over by the technician.

#### THE ORGANIZATION OF R.C.M.

The Director of the Experiment Station, Dr. Lyon, is the administrator of the Experiment Station R.C.M. organization and of the plantation activities of the Experiment Station staff.

In January 1936, Mr. Davis, associate chemist, took over the responsibility of attention to details coincident with the operation of the R.C.M. division of the chemistry department in Honolulu. Mr. Davis, senior member of the department research staff, also devotes a portion of his activities with the author and with Messrs. Yuen, Hamamura, Nishimura, and Chu to research problems leading to improvements and modifications in existing studies and in the development of new assemblies. Francis Fong and L. Kawamura are research assistants in the laboratory studies. C. Danner and A. Postl are engaged in stockroom and in shipping duties.

The author continues in the regular duties of department head, devoting about one-half of his time to plantation visits and to research studies in the field. Assistance in plantation visits is provided by Messrs. Hamamura and Nishimura, R.C.M. technicians.

In plantation field research the author cooperates with R. J. Borden, Agriculturist at this Experiment Station and with the four Island Representatives of the Agricultural and Genetics Departments—F. C. Denison, Oahu; O. H. Lyman, Hawaii; John W. Anderson, Kauai, and D. S. Judd, Maui. Further cooperation is offered by this group to the plantation staffs in field research and in associated problems. W. L. McCleery, Sugar Technologist, J. P. Martin, Pathologist, Dr. A. J. Mangelsdorf, Geneticist and their respective staffs also contribute to the progress of R.C.M. studies.

The Chief Clerk of the Experiment Station, A. R. Grammer, and his staff, F. D. Kennedy and F. W. Littlejohn, superintend business details. Office duties in the chemistry department which are associated with the R.C.M. organization are in charge of R. Boyen.

#### CORRELATIONS

The value of R.C.M. data is greatly increased by correlation with other independent means of determining the status of soil fertility and by correlation with

crop response and crop requirements. At the meetings of the International Congress of Soil Science (4), held in Oxford, England, in 1935, this subject received attention of several delegates at informal gatherings. The conflict of field experiment versus chemical analysis of soil was shown to be entirely out of line by some of the members, chiefly because no conflict should exist. Either means of soil evaluation it was believed, possessed merits and advantages not found in the other. Either method of arriving at nutrient requirements for any given agricultural land might at least supplement the other, it was agreed.

In our experiences in Hawaii we have found that a site selected for a field experiment or a Mitscherlich sampling station may be totally at variance with the average fertility of what appeared to be a very uniform portion of the field.

Due to the brevity of performing R.C.M. analyses and to the ease and rapidity with which a detailed survey may be accomplished in a field, an R.C.M. field study may prove a valuable preliminary step in selecting a representative location for a field experiment or Mitscherlich sampling station.

Another point in favor of correlating R.C.M. with other field studies embraces the value to accrue later to the agriculturist after such relationships have been established. A field experiment requires for completion a period equal to the age of the crop at harvest. The minimum time required for completing and reporting a Mitscherlich test is about 4 months. An R.C.M. survey may be completed in the course of a few days—certainly between harvest and the preparation of a field for the next crop (ratoon or plant). As to the wisdom of employing correlations rather than any single means of evaluating the supply of available soil nutrients in a field, we present the following paragraph by Mr. Borden:

It is our opinion that it would be unwise to build up a plantation fertilization policy on the analytical data secured from any one single measure of soil fertility. The present extensive application of the data secured from the rapid chemical method of testing soils has been made possible only through the weight and reliance placed upon the correlating of other independent means of evaluating the available nutrient status of our soils. To dispense with methods for verifying an opinion based on results from a single type of analyses, would not be in accord with the conservative policy of the Hawaiian sugar planter.

This subject has been discussed at some length in *Handbook of Hawaiian Soils* (5). Quoting from this publication:

Earlier studies by Stewart and McGeorge of the results of their citric acid soluble analyses of soils when compared with a response to phosphate and potash fertilizers applied in the sugar cane field, led them to suggest that (a) a response to applied phosphate fertilizers would not be expected if the citric soluble phosphate was above .004 per cent except on the more acid, upland soil types where the limit might be set a little higher, perhaps at .006 per cent, and (b) when dry land soils showed .04 per cent potash and wet land soils .05 to .06 per cent potash, little response would be obtained in the field from potash applications.

A summary of data from the more recent cooperative field experiments studied at the Experiment Station, H.S.P.A., would indicate that the above phosphate figures may still hold, but it suggests a slight change in the potash limits (Fig. 12). [See illustration on page 214.]

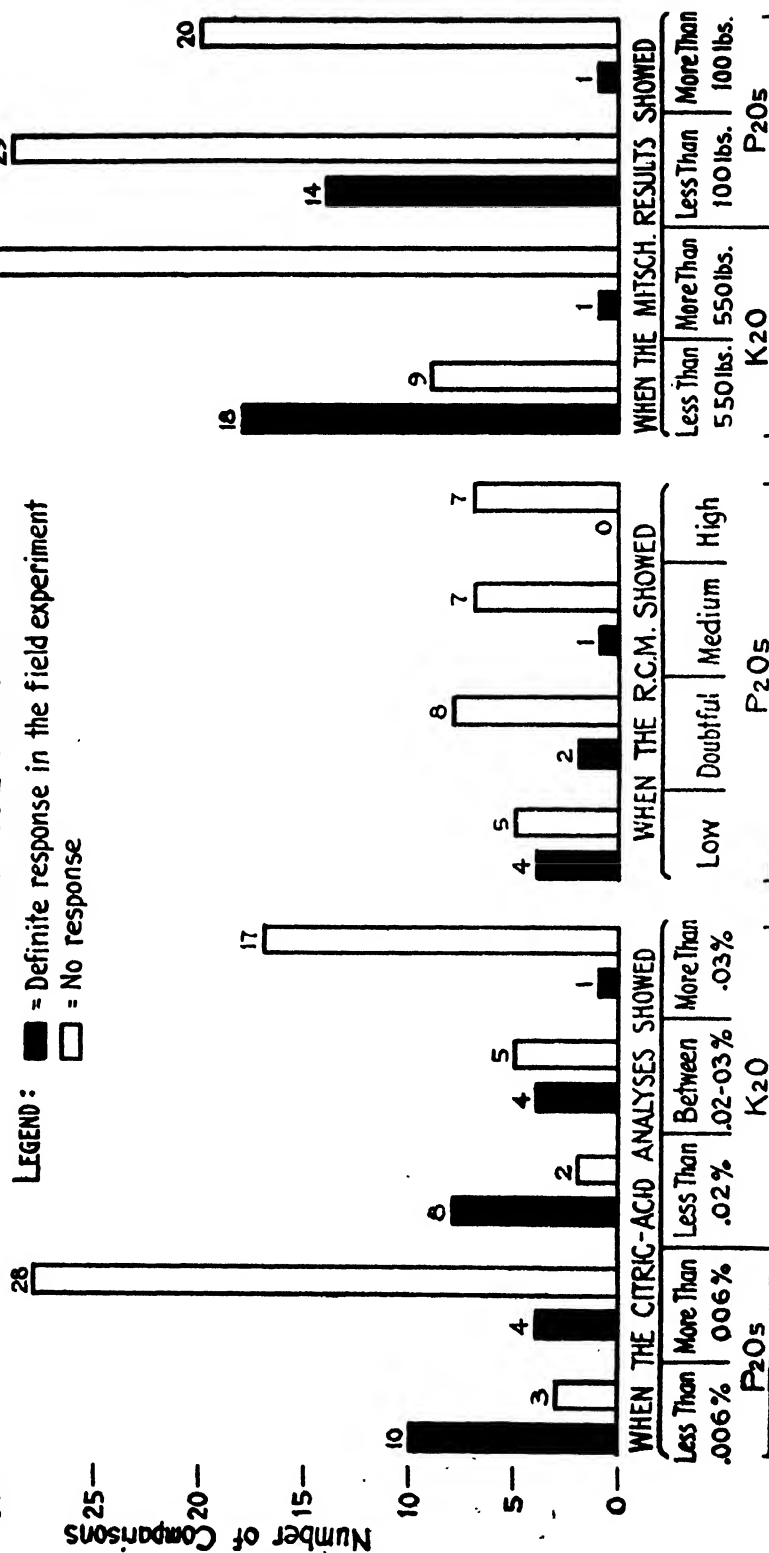
From forty-five recently harvested phosphate experiments, which are quite clearly interpretable as showing either a response or no response to phosphate applications and which are rather widely distributed throughout the Islands, we note the following correlation with citric soluble analyses of soils taken from the experimental areas:

(a) Thirteen soils with less than .006 per cent  $P_2O_5$ , ten of which showed a response to phosphate; ten of these thirteen soils with less than .004 per cent  $P_2O_5$ , eight of which showed a definite response to phosphate.



# SOME CORRELATIONS BETWEEN RESULTS OF FIELD TESTS AND SOIL ANALYSES

**LEGEND:** ☒ = Definite response in the field experiment  
☐ = No response



(The above illustration is Fig. 12 from the *Handbook of Hawaiian Soils*, Association of Hawaiian Sugar Technologists, page 93, 1935.)

(b) Thirty-two soils with citric soluble  $P_2O_5$  at .006 per cent or more, only four of which showed a response to phosphate applied.

From thirty-six good potash experiments from rather widely distributed cane areas, we note the following:

(a) Of six soils with citric soluble potash above .04 per cent, all showed no response to applications of potash fertilizer.

(b) Eleven soils with between .03 and .04 per cent  $K_2O$ , only one of which responded to applied potash.

(c) Nine soils between .02 and .03 per cent  $K_2O$ , only four of which responded to potash.

(d) Ten soils with less than .02 per cent  $K_2O$ , eight of which showed a definite response to potash.

The amount of data for a study of the correlation between the recently adopted rapid chemical analyses for soil nutrients, with the results secured in field experiments, is inadequate for the purpose of drawing definite conclusions at this early date. However, the data we have from thirty-four phosphate tests may be indicative of what we may expect:

(a) Nine soils showed *low* phosphate by the rapid chemical method (R.C.M.) and only four of these have responded to phosphate.

(b) Ten soils showed *doubtful* phosphate by the R.C.M. and two of these have responded to phosphate.

(c) Eight soils showed *medium* phosphate and one of these responded to phosphate.

(d) Seven soils showed *high* phosphate and none of these gave a response to applied phosphate.

Only nine potash experiments have been harvested, for which we have R.C.M. potash, and the results are not adequate for further discussion.

The correlation between the results of the Mitscherlich test with Sudan grass, and the response secured in the field experiment with sugar cane, are also indicated in Fig. 12. The data have been grouped into two divisions which indicate that either a definite response has been secured or that no response was obtained in the respective field test from which the soil was taken for the corresponding Mitscherlich analysis.

If we set our critical limit for potash at 550 pounds per acre-foot, we infer that we should get no response on soils that have more than this amount by the Mitscherlich measure, and conversely that we should get a definitely increased yield response on soils which have less than this critical limit. With this criterion, we note that our potash correlation is quite favorable, especially as regards a lack of response in the field when the Mitscherlich test has indicated more than 550 pounds of potash per acre-foot. In fact, it appears quite clear that we can reliably detect the soils that have an abundance of potash and upon which further application of potash fertilizer will hardly be justified. Our ability to find the low potash soils is not quite as good, but most of our disagreements come from the more alkaline soils and are probably due to some chemical change or pathological condition that operates differently in the Mitscherlich pots than in the field.

Setting up our critical phosphate limit at 100 pounds per acre and expecting thereby that soils with more than this amount will probably not respond to applied phosphate, and conversely that soils showing less than 100 pounds will show yield gains when phosphate applications are made, we find our data show a close agreement with the first expectation but not with the second. There are probably good reasons for this poorer correlation:

(a) *Pythium* root rot has been a factor in reducing yields in those Mitscherlich pots which have not received heavy applications of calcium phosphate and the low yields of these pots have undoubtedly been interpreted as due to low phosphate availability in the soil being tested.

(b) The indicator crop used may have been unable to extract its phosphate from a high phosphate fixing soil with sufficient speed to meet its needs during its short, rapid growth period in the pots.

(c) Frequently a field test may fail to show yield differences, due to plant food variations when some other limiting factor, such as drought, dominates the growing conditions in the field.

(d) Finally, the soil sample that was tested in the Mitscherlich pot may not have been a truly representative one. However, the encouraging feature of the phosphate correlation is the

fact that the Mitscherlich test can and does quite safely indicate where we may leave off phosphate fertilizers without reducing the cane yields.

With pineapples, Magistad had found that where there is 500 pounds per acre of replaceable potash in the soil the crop will not respond to potash fertilization. In 19 pineapple field experiments, 14 soils with more than 500 pounds of replaceable potash failed to show a response, while 5 soils that had considerably less (in fact less than 250 pounds) did respond to applications of potash fertilizer. Also, where soils have shown more than 25 parts per million (about 65 pounds per acre-foot) of readily chemical-soluble phosphorus, no response has been secured from phosphate fertilizer applied in the field.

Correlations with crops other than sugar cane and pineapples have not been definitely shown because the data are as yet inadequate.

Some correlation studies have been made between the various chemical methods for determining availability and also between chemical and biological methods. In general, the agreement has been quite good. It is doubtful, however, if the comparison of one method with another has the same value as the comparison of each method with the actual results secured in the field. Hence these methods' interrelations will not be discussed.

However, in the Director's Monthly Report for December 1936, Mr. Borden points out the relationships which appear to exist between soil pH and available nutrient supply. Included in these correlations are also R.C.M. data. Mr. Borden states:

Correlation studies from results of our 1935 soil tests at the Mitscherlich department have been made to show the relationship that apparently exists between the pH of the soil and its available supply of mineral plant nutrients. Since rapid chemical analyses were also made on these soils, our study includes both the R.C.M. and Mitscherlich measures of availability. A summary is offered as follows:

#### RELATIONSHIP OF SOIL pH AND AVAILABLE POTASH

No. of Tests	pH Grouping	Avg. Lbs. K <sub>2</sub> O per acre-foot by Mitscherlich	No. of Tests	R.C.M. Class	Avg. pH
42	5.0-5.4	320	94	Low	5.5
61	5.5-5.9	554	51	Doubtful	5.9
62	6.0-6.4	640	89	Medium	6.2
60	6.5-6.9	720	25	High	6.2
19	7.0-7.4	810			

#### RELATIONSHIP OF SOIL pH AND AVAILABLE PHOSPHATE

No. of Tests	pH Grouping	Avg. Lbs. P <sub>2</sub> O <sub>5</sub> per acre-foot by Mitscherlich	No. of Tests	R.C.M. Class	Avg. pH
42	5.0-5.4	46	36	Low	5.7
61	5.5-5.9	96	50	Doubtful	5.8
62	6.0-6.4	153	29	Medium	5.9
60	6.5-6.9	174	144	High	5.9
19	7.0-7.4	264			

The data as summarized indicate that the lower available supplies of both potash and phosphate are found associated with the more acid soil conditions.

The following article, "Soil Tests," is contributed by Mr. Borden. It is particularly appropriate to the important subject of correlations.

## SOIL TESTS

A large share of the present activities of a plantation agriculturist is concerned with the determination of the status of available plant nutrients in the soil, so that he may recommend an optimum fertilizer practice for fields, and even for parts of fields, that are being cropped. Considerable time is being devoted to sampling and to analytical work on these samples. The rapid chemical methods for analyzing soils, crusher juices, irrigation waters and plant materials are now a part of the routine procedure concerned with a field control of fertilization at several plantations.

The question that arises quite frequently now is, "Are these rapid chemical analyses sufficient by themselves, and can we now dispense with the biological soil tests such as the Mitscherlich test and the field experiment?" May we attempt an answer to this query?

Someone has said, "A diagnosis, like a criticism, is often the opinion of one man, and no one is infallible, however great his knowledge, experience, and up-to-dateness." The analogy is applicable in two ways here: no single test of the availability of plant nutrients in the soil is infallible; and no diagnosis will be apt to be correct every time, if it has been based on data secured by a single type of investigation and measurement.

We are expected to make a thorough study and to recommend a wholly conservative fertilization policy. Like the physician who makes his diagnosis and indicates his treatment only after he has taken both your temperature and pulse beat, and looked also at your skin, eyes, mucous membrane, and secretions; and like the steel-construction engineer who not only secures chemical analysis of his alloys, but physical measurements of density and tensile strength and even X-rays of his materials, and who then applies a test load to his job before turning it over as completed, we are in a better position to support our recommendations when they have been made both on chemical analyses of soil and of plant material grown thereon, and on the more rapid biological (*Mitscherlich*) tests of this same soil. Especially do we consider it advisable to test all large soil groups by the Mitscherlich test, whenever we are on the point of omitting phosphate or potash fertilizer from a large area, in order that we may verify the presence of an abundant supply which has been indicated by the rapid chemical analyses. Likewise, as a further safeguard, we would urge the installation of an occasional field test on areas that appear from our rapid chemical tests to be adequately supplied with a specific mineral nutrient. This test would be a very simple one, including plots both with and without perhaps 200 pounds of the plant food in question, installed with the idea of its being repeated through successive crops so that the first indication of an approaching deficiency in the particular soil might be secured. Thus it is quite probable that both the Mitscherlich pot test, and the simple "plant-food" field experiment, will have a real value in verifying a diagnosis of adequate phosphate and potash that has been made from results of rapid chemical analyses.

The agreement between indications of the fertility status in the soil as secured from the R.C.M. (rapid chemical method) and Mitscherlich method is not always identical; neither are the indications from field experiments and R.C.M., or from field experiments and Mitscherlich tests always in agreement, although the general

correlation is high and very satisfactory. Hence there is probably a real value in having results from *both* of the measures of fertility, when any material change in fertilizer policy is being planned. Only rarely have we found no response in the field experiment when *both* the R.C.M. and Mitscherlich tests have indicated a deficiency. However, we can cite specific instances which are exceptions as follows: (1) indications of a phosphate deficiency by the Mitscherlich test, but of an ample supply by the R.C.M. test of soil from a reliable field experiment that showed no response to phosphate; (2) indications of a potash deficiency by the R.C.M. analyses but of an ample supply by the Mitscherlich test on soil from a reliable field experiment that showed no response to applied potash; and (3) no response to phosphate in a field experiment, soil from which appeared to have a very low supply by both the R.C.M. and Mitscherlich tests. It is the occurrence of this occasional exception that warrants our belief that no one of these methods of testing soils can be given up entirely. Moreover, we have not found a single instance where a definite response has occurred in a "Grade A" field experiment when the soil analyses by *both* the R.C.M. and Mitscherlich test have indicated an adequate supply of either phosphate or potash. Thus when these two particular tests will verify each other in indicating an adequate supply, we can feel pretty safe about a field fertilization policy that omits one or both of these plant foods.

The field experiment is and, for some years to come, will undoubtedly be a very definite tool to use on those soils which have been tested by R.C.M. and found to be deficient, and it is doubtful if it can be dispensed with entirely. Because of errors connected with soil sampling, variations in climatic conditions throughout the sugar cane areas; the different plant food requirements of different varieties, and the difference in composition of irrigation waters, etc., it is a most difficult undertaking to attempt a thoroughly reliable quantitative interpretation, from either the R.C.M. or the Mitscherlich results, of the most economic amount of phosphate or potash that should be applied in fertilizers to soils which apparently have an inadequate supply. Thus, having located, by use of our quicker tests (R.C.M. and Mitscherlich), large soil areas that are deficient in one or both of these mineral plant foods, it will be essential that we install a well-planned "amounts" test thereon, in order to determine the most economic amount to apply on similarly cropped areas that have given us evidence of a similar availability of these mineral soil nutrients.

Apparently therefore, neither the Mitscherlich soil test nor the field experiment will be wholly supplanted by the rapid chemical methods of soil analyses. Rather will we use all three of these valuable measures of soil fertility, correlating their results on certain key areas, and thereafter making a much wider application of the findings from the field experiment to smaller areas which are shown by the rapid chemical tests to have a similar nutrient status. Thus, plans for a differential fertilization will be on the soundest possible basis that is available at this time.

#### A DISCUSSION ON METHODS OF CHEMICAL ANALYSES

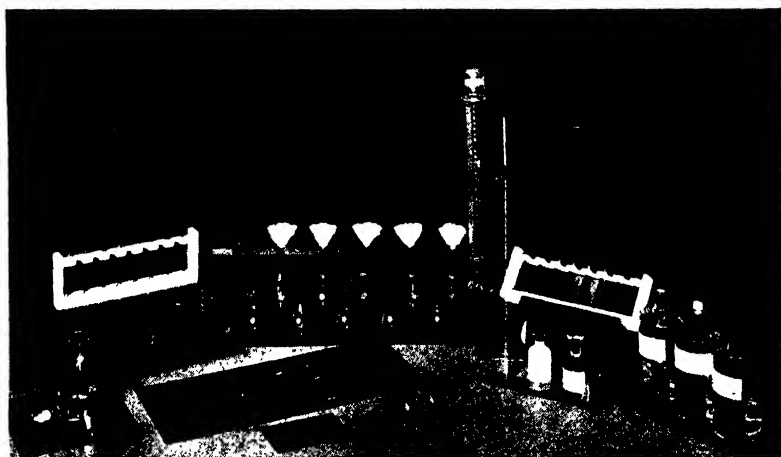
The portion of the paper immediately following is devoted to a brief exposition of the chemical methods of analysis employed in R. C. M. studies. It, in turn, will be followed with a listing of reagents by number or other designation and will include a description of the chemical composition of each reagent.

At present the full complement of R. C. M. consists of determinations of :

Available nitrogen in soil	Phosphate in boiler water
Total nitrogen in soil	Phosphate in filter cake
Total nitrogen in filter cake	Readily soluble potash in soil
Total nitrogen in mill water	Potash in crusher juice
Total nitrogen in crusher juice	Potash in molasses
Total nitrogen in plant material	Potash in irrigation water
Total nitrogen in molasses	Potash and phosphate in mill ash
Readily soluble phosphate in soil	Calcium in soil
Phosphate fixation in soil	Calcium in filter cake
Phosphate in crusher juice	Soil reaction

*Available Nitrogen in Soil:*

Soil-extracting mediums (acidulated salt solutions) used in the potash and phosphate determinations are not satisfactory in available nitrogen work due to the color imparted to the extracts by organic matter dissolved from many Hawaiian soils. Water alone as an extractant, without the addition of electrolyte, is also unsatisfactory



Rapid Chemical Method for the determination of available nitrogen in soils.

due to the tendency of many local soils to deflocculate when thus shaken. This makes filtration by ordinary means difficult, if not impossible. A dilute potassium sulfate solution was finally selected for extracting available nitrogen from the soil specimens.

A measured amount of soil is extracted with a definite volume of the reagent. After a maceration period of one minute, the mixture of soil and extracting solution is filtered. The filtrate is analyzed separately for ammoniacal and for nitrate nitrogen. The amount of "available" nitrogen is the sum of the two forms.

*Ammoniacal Nitrogen:* Five ml. of the filtrate are transferred to a 3-dram narrow shell vial for the ammoniacal nitrogen test. To this aliquot of the filtrate is added 4 drops of an alkaline tartrate solution, followed by the addition of 1 ml. of a modified Nessler reagent. The alkaline tartrate prevents precipitation in the test

## EXPLANATION OF PLATE I

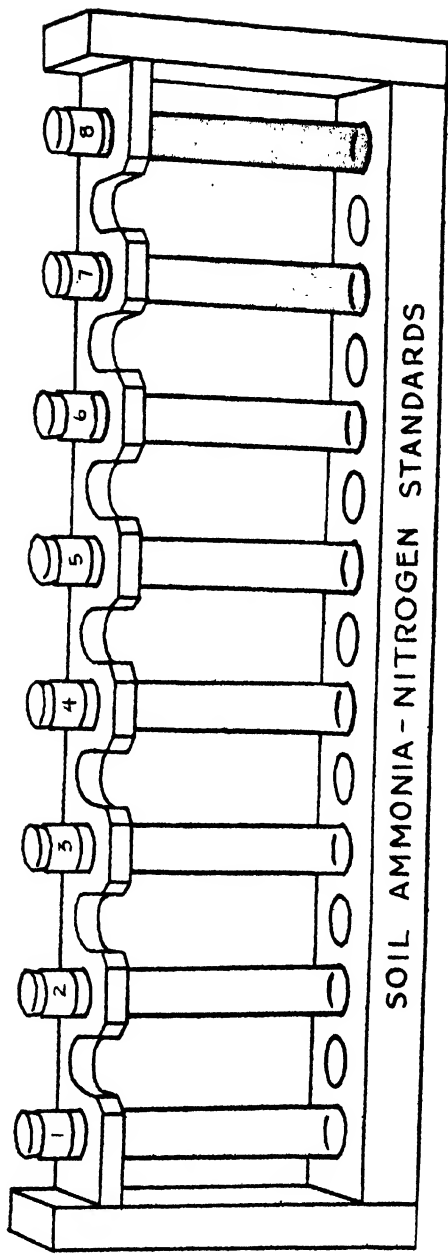
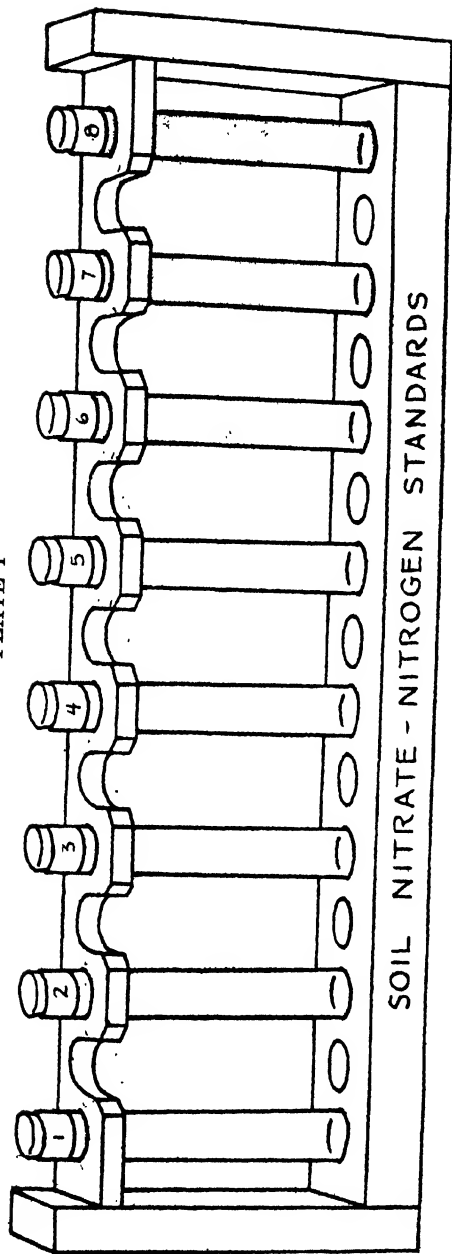
### *Soil Nitrate-Nitrogen Standards*

The principle followed in this set of standards is similar to the others. The tubes, eight in number, increase progressively from a pale to a deep yellow. Legends appear on these tubes giving the percentage and pounds per acre-foot of nitrate nitrogen. The analyst, therefore, can jot down his findings immediately after comparing his unknown with the standard in this rack before the window of the illuminator. The numbers indicated have been placed there only for purposes of illustration and do not appear on the standards themselves.

### *Soil Ammonia-Nitrogen Standards*

The colors increase progressively in intensity from Tube 1 to Tube 8. Percentage and pounds per acre-foot of ammonia nitrogen are indicated on each one of these standards. A more detailed discussion of all the standards will be found in the text.

PLATE I







vial of alkaline earths, chiefly magnesium and calcium, which may be extracted from some of our lowland soils. The Nessler reagent causes the formation of a complex compound of mercury and ammonia which is indicated by a light orange-yellow in the lower concentrations of ammoniacal nitrogen, and by darker shades for larger amounts until in the highest concentrations a brownish-orange precipitate is formed. The vial is stoppered and thoroughly shaken to disperse any precipitate that may have formed and is then matched against standards which are placed in a sliding rack mounted upon an illuminating device. The result, expressed both in per cent nitrogen (ammoniacal) and as pounds nitrogen per acre-foot, is indicated on the vials containing the color standards.

*Nitrate Nitrogen:* After the ammoniacal nitrogen content of the soil extract is determined, 25 ml. of the filtrate are returned to the original beaker with a pipette, after discarding the excess. This beaker containing the 25 ml. is then placed upon a hot plate and the solution evaporated nearly to dryness. Two ml. of phenoldisulphonic acid are then added to the residue and the mass disintegrated by mixing with a stirring rod. After a period of standing, to insure complete reaction of the reagent with the nitrate, the mixture is diluted with water and neutralized with concentrated aqueous ammonia. If nitrate nitrogen is present a yellow color will result, the intensity of which will vary with the nitrate content of the soil extract.

The yellow liquid is then transferred to a shell vial identical with those containing the color standards. Comparisons are then made with standards which are held in a sliding rack mounted on an illuminating device. The per cent of nitrate nitrogen and pounds nitrate nitrogen per acre-foot are expressed on the vials containing the standards.

#### *Total Nitrogen in Cane Juice, Plant Material, Mill By-Products, and Soil:*

The presence in these substances of nitrogen in organic combinations necessitates treatment more severe than that used for the determination of "available" soil nitrogen. The employment of the regular laboratory or Gunning method, without modifications, is out of the question in a scheme of rapid analysis. Costly installation of heavy apparatus makes this regular laboratory method prohibitive. It is also quite essential that a trained analyst perform the conventional laboratory determination.

Thus the problem became one of obtaining the nitrogen in the cane material in a form which could be accurately determined by R. C. M. technic with the simplest procedure and equipment. In general, when an organic substance containing nitrogen is digested with concentrated sulfuric acid at the boiling point, the carbonaceous material is destroyed and the nitrogen is converted into ammonium sulfate. After completion of this process, the solution is cooled and diluted with water. When the diluted solution is made alkaline with an excess of sodium hydroxide, ammonia is liberated. Upon heating, the ammonia is distilled into a trap containing dilute acid. Sulfate of ammonia is formed if the receiving acid medium is dilute sulfuric acid. In the usual laboratory analysis the absorption is effected in standardized acid, the excess acid remaining in the receiving flask after distillation being titrated with a standard alkali solution. In this manner the total nitrogen in the sample is determined.

The rapid method follows the principle indicated above. While in the regular laboratory procedure, cumbersome apparatus are required, the rapid method employs only equipment of the simplest design. Details have been worked out so that only a minimum of attention is required. The digesting reagent of concentrated sulfuric acid is prepared from a pure grade of material, free from nitrogen, and to it is added selenious oxide to hasten (catalyze) the decomposition of the organic sample. The usual bulky distilling and condensing apparatus gives way to an easily attached connecting bulb and tube, the latter discharging into a calibrated trap of dilute sulfuric acid cooled by a water bath. (See illustration.) Titration, too, is eliminated by simple colorimetric determination of the (ammonia) nitrogen in the dilute acid



Rapid Chemical Method for the determination of total nitrogen in cane juice, plant material, mill by-products and soil.

solution in which the distillate is collected. The entire range of the determination is adjusted so that the Nesslerization of an appropriate aliquot of the distillate gives a color falling within the limits of the series of graded ammonia color standards.

The analyst is thus relieved of the exacting detail of titration or of the cumbersome manipulation usually required in Nesslerization tests, yet without sacrifice of accuracy (as was determined in an exhaustive study). Thus is effected an appreciable saving in time and the necessity for specialized training is eliminated.

The determination of total nitrogen in soil and in dried plant material requires the use of an analytical balance where extreme accuracy is desired. With the advancement on many of the plantations to special research studies, balances have been added to the equipment. However, measured portions of soil and dried plant material, when pulverized in a mortar, may be employed in studies of total nitrogen content where comparisons are to be made of a relatively large number of samples having practically identical physical characteristics. In such cases calibrated measuring devices supplant the balance.

The determination of total nitrogen in crusher juices is made upon prepared

specimens withdrawn by standard pipettes. Fortunately the two procedures most frequently used (available soil nitrogen and nitrogen in crusher juice) require neither balance, titration nor analytical skill. Either determination may be made in one hour's time.

The procedures for the determination of total nitrogen in cane juice, plant materials, mill by-products and soils vary in details as to the handling of each individual substance (see directions). However, the principle is essentially the same in all 4 analyses. The total nitrogen determination approximates closely that obtained by the Gunning method but the procedure requires much less time and is equally as accurate.

The sample to be analyzed is digested in a 300-ml. Kjeldahl flask with a measured amount of concentrated sulfuric acid containing a mineral catalyst (selenium oxide) and a small amount of potassium sulfate. The latter is added for the purpose of raising the boiling point of the mixture. An electric hot plate of special design is used to effect the digestion. The hot acid oxidizes the carbonaceous matter contained in the sample and converts the organic nitrogen into an inorganic form. When the digestion is completed, the mass is cooled. It is then diluted with distilled water.

In the analysis of plant materials, mill by-products and soils, the solution is transferred at this point to a 250-ml. volumetric flask, cooled and made up to volume. Following a thorough mixing, a 10-ml. aliquot is removed for distillation. In the analysis of cane juice, the entire solution contained in the Kjeldahl flask after digestion and dilution is used for the subsequent distillation.

To the original Kjeldahl flask in cane juice analysis, or to a clean Kjeldahl flask containing 10 ml. of the diluted solution, is added distilled water and a measured amount of a strong solution of sodium hydroxide. The flask is then connected to a trap leading to a receiving test tube holding a very dilute acid. The test tube is immersed in a flask of cold water, which acts as a condenser. Heat is applied to the Kjeldahl flask. Ammonia, liberated by action of the alkali, is distilled over and collects in the weak acid. After distillation has been completed, the distillate is cooled and made up to a prescribed volume. An aliquot is then transferred to a 3-dram shell vial and the test completed as in the determination of available ammoniacal nitrogen in soil. The addition of Nessler reagent causes the characteristic reaction which produces the coloration in the tube. The tube is then matched with the color standards, the results being obtained by reference to a table.

#### *Nitrogen in Irrigation and Mill Waters:*

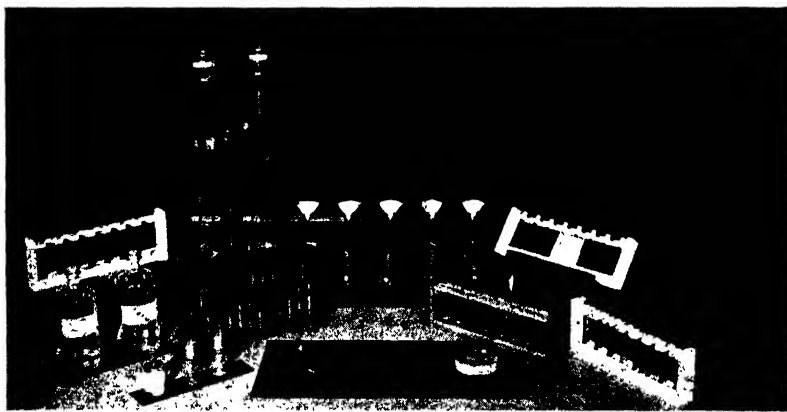
Nitrogen in irrigation water may consist of either one or more forms, ammoniacal, nitrate and/or organic. Most irrigation and drainage waters contain only ammoniacal and nitrate nitrogen. These waters may be analyzed by the procedures which follow closely the method for determining available nitrogen in soil. A sample of the water is tested directly with Nessler reagent for ammoniacal nitrogen. Another aliquot is evaporated and the phenoldisulphonic acid method is used to determine the nitrate nitrogen content.

For mill water or water to which filter cake has been added, the total-nitrogen method is employed. Concentrated sulfuric acid reagent and potassium sulfate are

added to an aliquot in a 300-ml. Kjeldahl flask. The mixture is placed on the special heater. Water is evaporated off and the organic matter is decomposed. The boiling sulfuric acid converts the organic nitrogen into the ammoniacal form. Following completion of the digestion, the residue is diluted and concentrated alkali is added. The ammonia is distilled into a dilute acid trap. The distillate is then analyzed by the regular ammoniacal nitrogen procedure. By referring to a table the p.p.m. total nitrogen and pounds total nitrogen per million gallons of water are obtained.

#### *The Determination of Readily Soluble Phosphate in Soil:*

A 10-gram portion of soil specimen from a calibrated metal cup is placed in a 125-ml. Erlenmeyer flask. To the soil in the flask is added a measured amount of a reagent which consists of dilute hydrochloric acid solution. The soil and reagent are then shaken for one-half minute and immediately filtered into a 50-ml. beaker. Ten ml. of the filtrate (soil extract) are pipetted from the beaker into another 50-ml. beaker and treated with several drops of concentrated nitric acid delivered from a dropping bottle provided for this purpose. The beaker with its contents of soil extract and nitric acid is transferred to a low-heat electric hot plate and evaporated to dryness, whereupon several drops each of concentrated hydrochloric and nitric



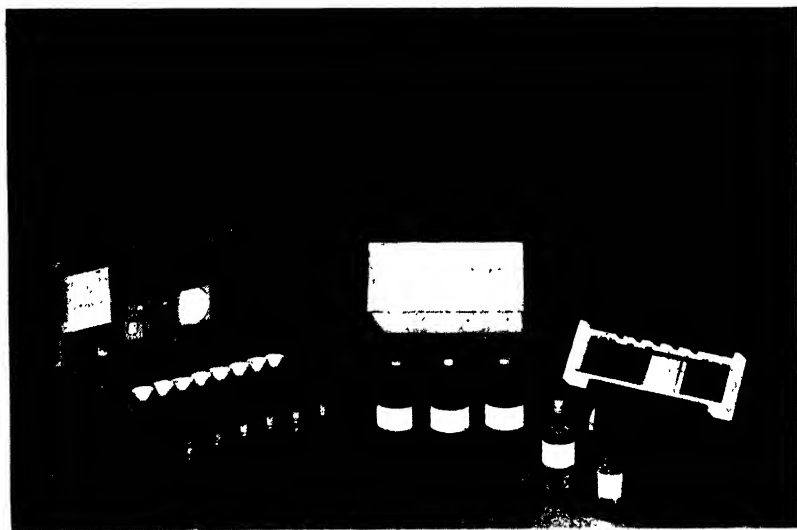
Rapid Chemical Method for the determination of readily soluble phosphate in soil.

acids are added and the extract again evaporated to dryness. As a result of the hydrochloric-nitric acid treatment organic substances which might otherwise interfere with the subsequent color development are destroyed and a light colored residue is obtained. The beaker containing the residue is cooled and to it are added 10 ml. of a reagent consisting of a carefully prepared dilute hydrochloric acid solution of ammonium molybdate. When the residue is dissolved by the reagent, the solution is transferred to a tall, narrow, phosphate comparison tube. Two drops or more of a reagent consisting of stannous chloride dissolved in dilute hydrochloric acid are added to the tube containing the test solution, whereupon it is well mixed by closing the tube with the thumb and inverting the tube several times. Immediately following the full development of the blue color resulting from the reaction between the

stannous chloride solution and the phosphate molybdate compound, the tube is transferred to an open groove in a sliding rack which holds a series of color standard tubes. This rack is mounted on an illuminating box devised for this type of analysis. The intensity of the color developed is approximately proportional to the amount of phosphate extracted from the soil. The color tube is compared with standards which are marked "High," "Medium," "Doubtful," and "Low," and the results are recorded accordingly. By referring to a data sheet which accompanies the assembly, one may obtain the percentage of  $P_2O_5$  in the soil specimen or the concentration in terms of pounds of  $P_2O_5$  per acre-foot of soil. In addition to the above-mentioned set of standards, another series is provided for the estimation of amounts of  $P_2O_5$  greater than that indicated by the "High" standard represented in the regular color standard set.

#### *The Estimation of Soil Phosphate Fixation:*

This procedure is used in order to determine the relative degree to which soils are capable of absorbing applied soluble phosphates. A phosphate solution of def-



Rapid Chemical Method for the estimation of phosphate fixation in soil.

inite concentration is shaken with a prescribed amount of soil and filtered after a specified period of contact. The amount of phosphate remaining in solution is indicated by the intensity of the blue color developed by the reaction of phosphate with the ammonium molybdate and the further reaction with stannous chloride. The resulting blue color is matched against standards contained in sealed vials and the measured degree of fixation is expressed in arbitrary numerical indices from 0 to 90 to which are prefixed the serial numbers of the phosphate fixation solution used in the tests.

The fixing solutions, adjusted to neutral reaction, contain 100, 500 and 1000 parts per million phosphate and are designated respectively as solutions Series 100,

## EXPLANATION OF PLATE II

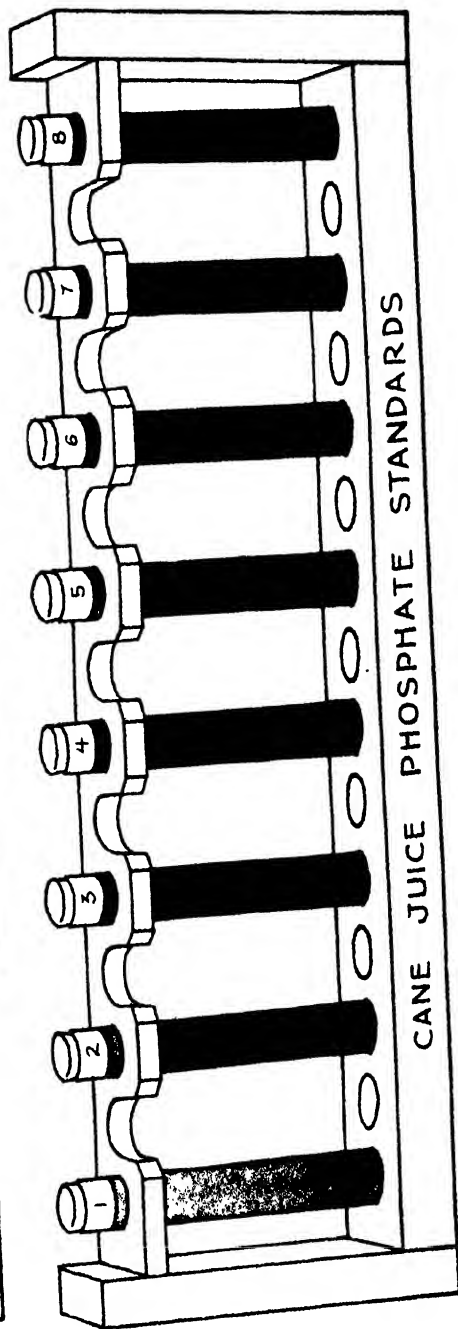
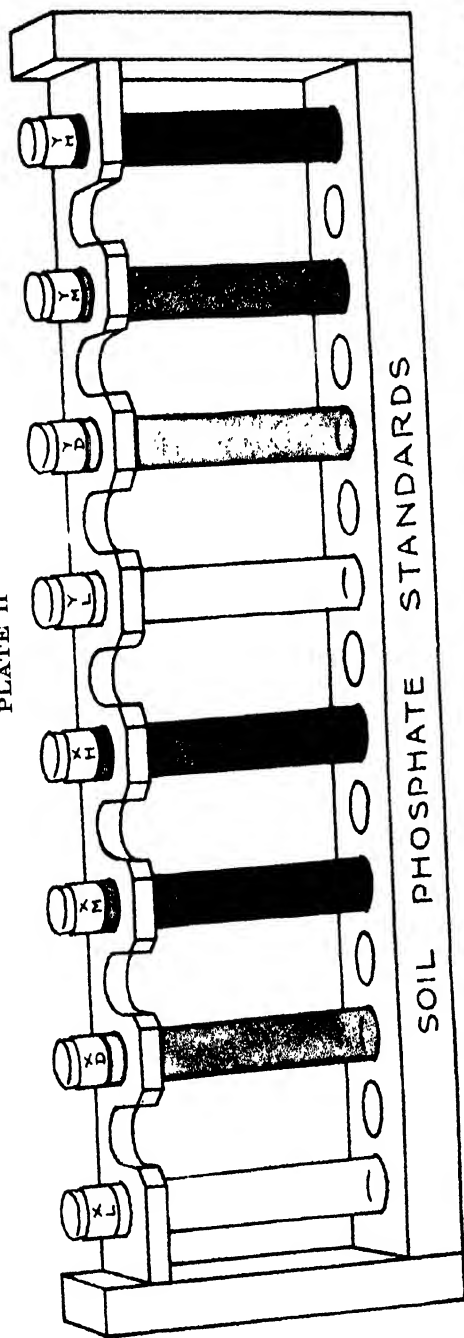
### *Soil Phosphate Standards*

The tubes shown are placed in the stationary positions in the rack, the unknown samples being compared in the notched intervening spaces. The legend "X" indicates a type of blue coloration which may be developed in normal or water-white soil extracts. The legend "Y" indicates a green-like blue coloration which is produced in the analysis when the soil extract is somewhat yellow because of ferric iron which it may contain. The letters "L," "D," "M" and "H" represent, respectively, low, doubtful, medium and high concentrations of the nutrient.

### *Cane Juice Phosphate Standards*

The numbers refer to a progressively increasing depth of color produced in the test, the matching of the unknown being made by comparison in the notched spaces provided in the rack before the light of the phosphate illuminator.

PLATE II



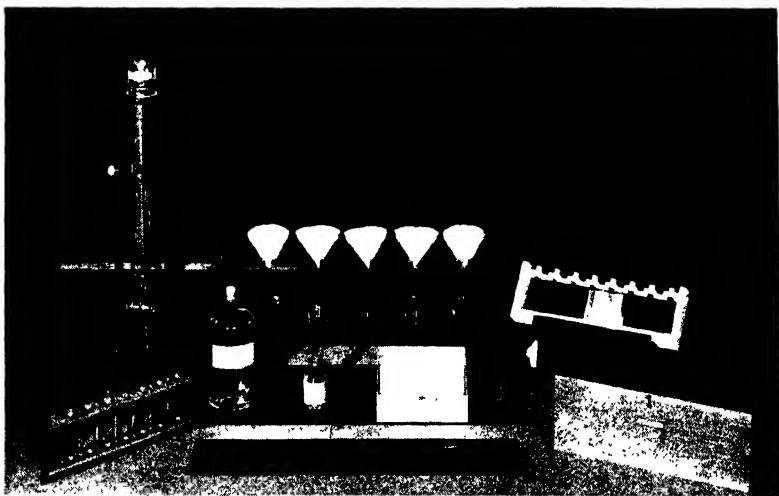




500 and 1000. Using these solutions at the specified ratio of 30-ml. solution to 5 grams of soil, tests may be performed equivalent to treatments with 1500, 7500 and 15,000 pounds  $P_2O_5$  per acre-foot of 2.5 million pounds of air-dried soil. These solutions are buffered to minimize the tendency of many of our Hawaiian soils to deflocculate when in aqueous suspension.

In making the tests, 5 grams of air-dry soil are measured in a calibrated metal cup and transferred to a 2-ounce graduated bottle. Then 30 ml. of Series 100 fixation solution are added. The mixture is shaken for 1 minute and then allowed to stand for 24 hours. At the end of this period it is reshaken for another minute, then the liquid and soil are separated by filtration. The filtrate is collected in a 3-dram, tall vial until it has been filled to about two-thirds of its capacity. One ml. of ammonium molybdate is added and the solution is thoroughly mixed. Then 1 drop of stannous chloride reagent is introduced and the solution is again mixed. A blue coloration will be produced in the test solution if phosphate remains in the filtrate. This blue color is matched against the standards. If the index obtained by this test is "60" or above, fresh portions of the soil may be retested, using the more concentrated solutions.

Two 5-gram portions of soil are transferred separately to bottles containing 30 ml. each of the solutions Series 500 in one case and Series 1000 in the other. Soil and solutions are shaken for 3 minutes, allowed to stand 24 hours, reshaken for 3 minutes, and finally allowed to stand for another 24 hours. At the end of this 48-hour period the mixture is shaken for another 3 minutes and filtered. The color test for these filtrates is performed in the same manner as for the Series 100 solution. The numerical indices are determined and tabulated with the prefixes of the series numbers of the fixation solutions.



Rapid Chemical Method for the determination of phosphate in cane juice.

#### *The Determination of Phosphate in Cane Juice:*

The sample of cane juice for analysis is filtered into a clean beaker. By using a special 0.5-ml. pipette graduated in 0.1 ml., a 0.3-ml. portion of the filtered cane

## EXPLANATION OF PLATE III

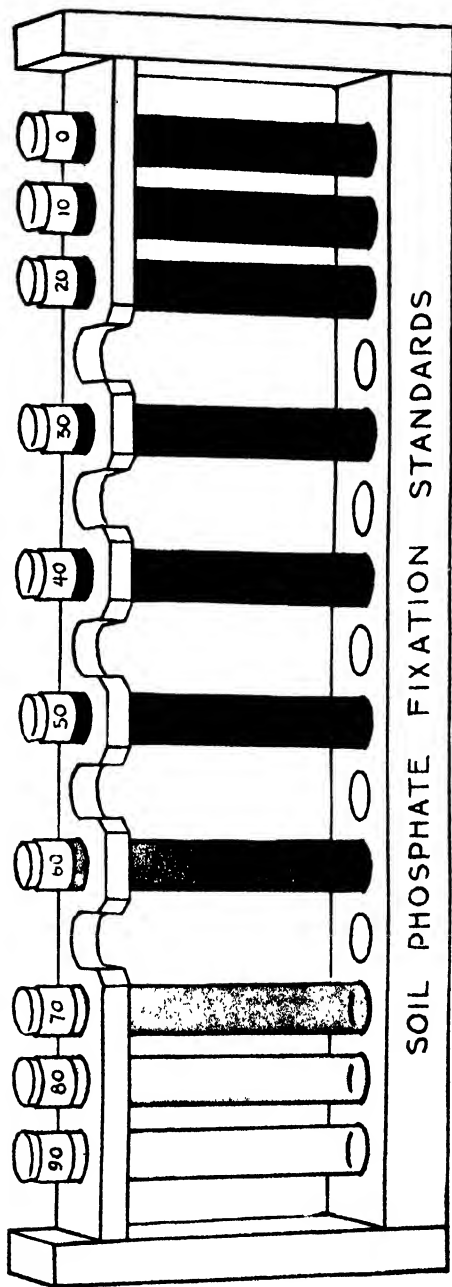
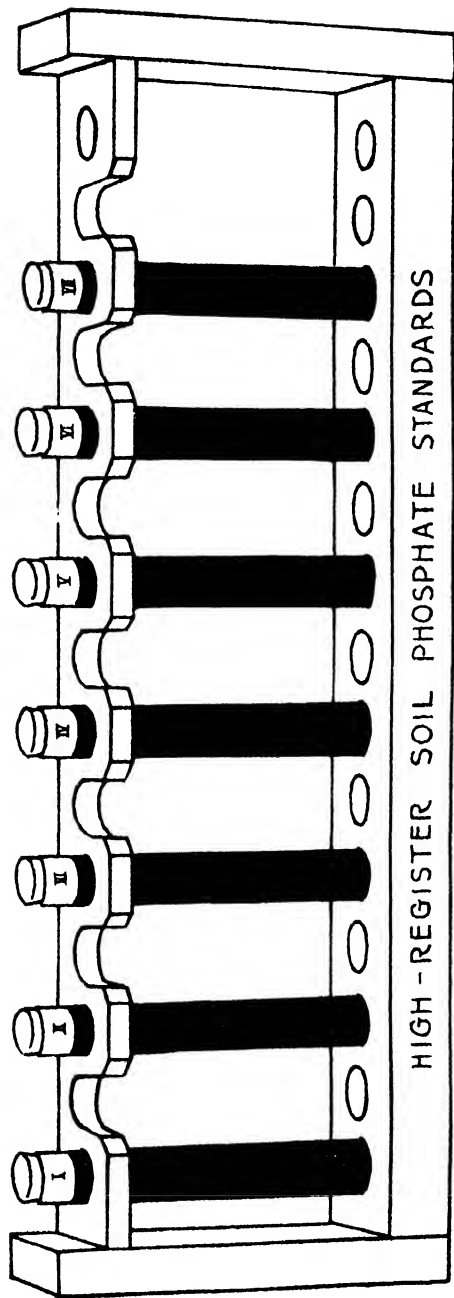
### *The High-Register Soil Phosphate Standards*

These standards were developed to enable the analyst to obtain more specific results where the concentrations of soil phosphate went beyond the limit of the "High" value in the regular standards. Here the more intense shades of blue are separated in gradual stages in seven steps and in this case the standards each carry a Roman numeral, increasing progressively with the depth of color in addition to a percentage and pounds per acre-foot figure of the corresponding concentration of phosphate as it exists in the field from which the soil was taken.

### *Soil Phosphate Fixation Standards*

This series of color standards is divided in depth of hue of coloration, the very palest discernible blue having been given an index number of 90, the depth of blue color becoming progressively deeper until a zero is reached, the latter being the deepest color tube in the set. The reason for using 90 to indicate the faintest blue color was originally intended as an indication of fixation of applied phosphates at 90 "per cent" or higher, and a test giving a color comparable to the zero tube, or full blue, would have passed through the analysis without having any phosphate fixed. Hence, roughly, phosphate fixation would have been negligible.

PLATE III





juice is transferred to a phosphate comparison tube, whereupon a solution consisting of dilute hydrochloric acid and ammonium molybdate is added until a definite volume is obtained. The color is developed with 1 or 2 drops of a stannous chloride solution and compared with a set of color standards prepared for cane juice analysis. When the color developed is darker than the darkest tube, smaller aliquots of either 0.2 ml. or 0.1 ml. are taken for analysis and conversely, when the color is lighter than the lightest tube, larger aliquots of either 0.4 ml. or 0.5 ml. are treated and compared. The aliquot of juice taken and the number on the color standard with which the unknown was matched, when referred to a data sheet, will indicate the percentage of  $P_2O_5$  by volume in the juice sample.

#### *The Determination of Free or Soluble Phosphates in Boiler Water:*

The collected sample is passed twice through filter paper to separate all precipitated phosphates from free phosphates. Two ml. of filtrate are transferred to a 3-dram, tall vial to which is added a solution consisting of dilute hydrochloric acid and ammonium molybdate until a definite volume is obtained. By the addition of 1 or 2 drops of stannous chloride solution a blue coloration is produced which is compared with the juice phosphate color standards on an illuminator. The concentration of free phosphates in parts per million is obtained by reference to a data sheet provided for this purpose.

#### *The Determination of Phosphate in Filter Cake:*

A weighed amount of filter cake is extracted with a measured quantity of a one-half normal solution of hydrochloric acid. To an appropriate aliquot of the extract, contained in a shell vial, is added a dilute acid solution of ammonium molybdate. The solution is then mixed and stannous chloride reagent, which develops a blue coloration, is added. The unknown is then matched against the color standard tubes used for the phosphate in cane juice determinations. The tube matched is referred to a table from which the results obtained are expressed as per cent  $P_2O_5$  and as pounds  $P_2O_5$  per ton of filter cake.

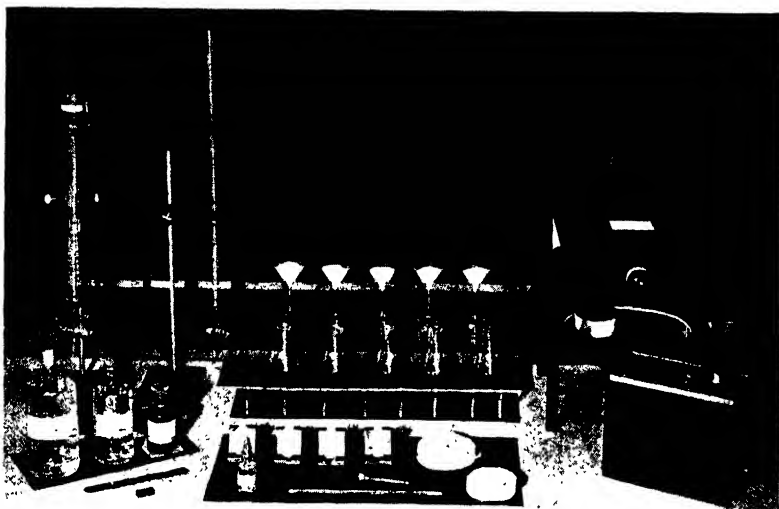
#### *Determination of Phosphate in Mill Ash:*

A 1-gram sample of mill ash is weighed on an analytical balance and placed in a 125-ml. Erlenmeyer flask. To the flask is added a measured amount of dilute hydrochloric acid. The flask is then shaken for 3 minutes and the solution immediately filtered into a 100-ml. beaker. Ten ml. of the filtrate (or extracted solution) are pipetted into a 50-ml. volumetric flask which is then filled up to its 50-ml. mark with distilled water, stoppered and thoroughly mixed. By means of a special 0.5-ml. pipette, graduated in 0.1 ml., a 0.3-ml. portion of the well-mixed solution in the flask is transferred to a phosphate comparison tube. The solution in the tube is then made up to a definite volume with a reagent consisting of a dilute hydrochloric acid solution of ammonium molybdate. One or 2 drops of stannous chloride solu-

tion are then added and the color thus developed is compared with the set of color standards used in the R.C.M. phosphate procedure for cane juice. When the color developed is without the range of the standards, a suitable aliquot of the diluted solution is taken and the comparison again made. This process is repeated, if necessary, until a good match is obtained. By referring the amount of solution taken and the number on the matching color standard to a printed data sheet, the percentage of  $P_2O_5$  in the mill ash is obtained.

#### *The Determination of Readily Soluble Potash in Soil:*

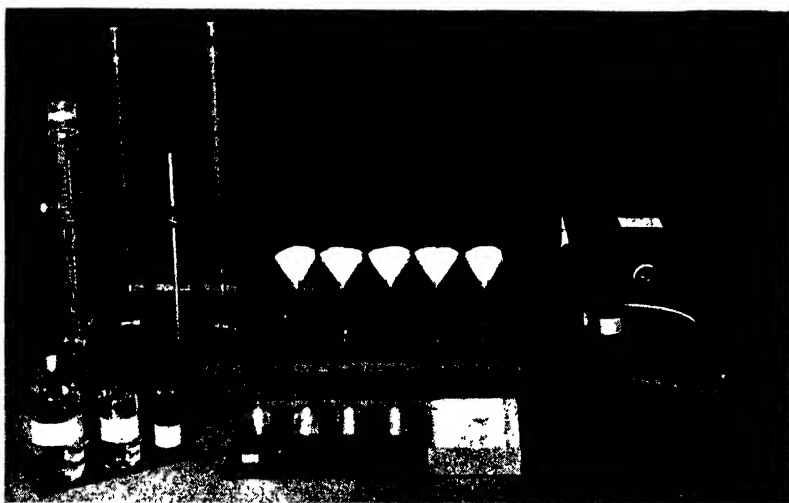
The soil specimen to be analyzed is transferred from a calibrated metal cup, holding 2.5 grams, to an Erlenmeyer flask of 125-ml. capacity. To the flask is added a measured amount of a reagent acidulated with nitric acid. The soil and reagent



Rapid Chemical Method for the determination of readily soluble potash in soil.

are agitated for one-half minute whereupon the mixture is poured into a previously prepared filter, the filtrate (soil extract) being collected in a 50-ml. beaker. One ml. of the soil extract is pipetted from the beaker and transferred to a flat-bottom comparison tube. Four drops of a reagent, consisting of a carefully standardized solution of cobaltous nitrate and sodium nitrite in acidulated distilled water, are now added. The comparison tube with its contents of soil extract and reagent is gently agitated for a brief interval and then placed in the recess of an inclined support. Immediately thereafter 1 ml. of an alcoholic reagent is carefully introduced along the inclined inner surface of the tube, forming a clear supernatant layer above the mixture of soil extract and reagent. The comparison tube, with its contents, is then carefully transferred to the rotor of the Experiment Station electric (or spring-driven) potash rotator. As a result of the combined oscillating and rotating action of the rotor in its orbital swing, the contents of the comparison tube are subjected to a standardized and peculiarly characteristic motion which results in the develop-

ment of a turbidity, the extent of which is directly proportional to the amount of potash extracted from the soil specimen. The rotating device is operated for exactly one-half minute. The comparison tube is then immediately transferred to the slot of the illuminating instrument prepared for this type of analysis. The degree of turbidity is determined by sighting vertically down through the column of liquid in the comparison tube, gauging the turbidity by the appearance or non-appearance of a number of parallel ruled lines graded in intensity from very faint to very heavy in a luminous field over a ground glass background. The various series of parallel lines are numbered 1, 2, 3 and 4. The operator refers the number of the group of lines which he can just distinguish to a data sheet which accompanies the assembly and from which he may read off directly the percentage of potash in the soil specimen, or its concentration in the field in pounds per acre-foot.



Rapid Chemical Method for the determination of potash in cane juice.

#### *Determination of Potash in Cane Juice:*

The juice collected for analysis is passed through a coarse filter paper. A preliminary test to determine the dilution necessary is conducted by adding, in a flat-bottom comparison tube, 1 drop of the juice to 1 ml. of a buffered reagent which consists of a solution of sodium acetate in dilute nitric acid. The procedure is repeated for a second vial, using 2 drops of juice. To each tube 4 drops of a standardized reagent consisting of a solution of sodium nitrite and cobaltous nitrate are added. The contents of the tubes are mixed slightly, and 1 ml. of the alcoholic reagent added along the inner wall of the inclined vial. The vials are then placed in the potash rotator and shaken for exactly a half minute. Readings are made on the standard potash illuminator. The technic following the dilution of the sample is carried out in exactly the same manner as that used in the soil potash assembly. Readings of the ruled potash chart are similarly made. For all the possible readings a table is supplied as a part of the procedure, giving the recommended dilution to be made for the regular analysis of the sample. These final dilutions are made by



pipetting out 1 ml. of the juice and adding the volume of the diluent determined by the preliminary test. One ml. of the diluted juice is transferred to a comparison vial, the necessary reagents added, the sample rotated and the readings taken as before. The percentage of potash in the juice is read off directly from a table which accompanies the assembly.

#### *The Determination of Potash in Irrigation Water:*

A measured volume of water is evaporated to dryness in a 400-ml. beaker. The potash in the residue is taken up in 10 ml. of a solvent consisting of sodium acetate and dilute nitric acid. After thoroughly polishing the beaker, the solution is filtered into a 50-ml. beaker. Fractional parts of a ml. of this filtrate are placed in potash vials and made up to a full ml. with the potash solvent. The regular potash reagents



Rapid Chemical Method for the determination of potash in irrigation water.

are then added. This is followed by shaking in the potash rotator. Readings are made on the potash illuminator in the usual manner. By referring these readings to a table accompanying the assembly, the concentration of potash is indicated directly in terms of parts per million, or pounds  $K_2O$  per million gallons. These figures are given under the proper column of potash solvent used in taking up the residue after evaporation of the water sample and the fractional part of a ml. of the extracted filtrate employed in the analysis.

#### *The Determination of Potash in Mill Ash:*

A weighed portion of a representative and pulverized sample of mill ash is treated for 1 minute with 50 ml. of a reagent consisting of sodium acetate and dilute nitric acid, and the mixture is then filtered. Fractional parts of a ml. of the filtrate are transferred to the regular potash comparison tubes and the volume made up to a full ml. with the potash solvent. Four drops of a reagent consisting of sodium

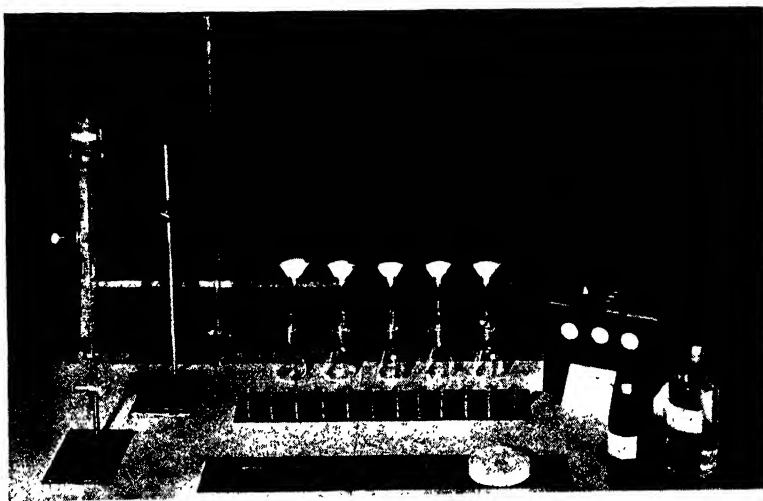
nitrite and cobaltous nitrate are then added and quickly incorporated. One ml. of an alcoholic reagent is allowed to flow into the tube with a minimum amount of mixing. The vial is then put into the potash rotator, shaken for half a minute, and the readings taken on the potash illuminator in the same manner as for soil potash. When the correct aliquot has been used the reading falls within the limits of the ruled chart. By referring to a special table, the potash content of the ash is read directly in terms of per cent  $K_2O$ .

#### *Determination of Potash in Molasses:*

A weighed sample of molasses is diluted to the point where its potash content approximates that of a representative specimen of cane juice. The solution is then filtered. It is possible to proceed from this point with an aliquot of the filtrate precisely as if it were filtered cane juice. Accordingly, the analyst is referred to the directions for the rapid estimation of potash in cane juice. A table applicable to potash in molasses has been prepared, taking into account the weight of the sample and the dilution. The results are expressed in terms of percentage and pounds of  $K_2O$  per ton of molasses.

#### *The Determination of Readily Soluble Calcium in Soil:*

Air-dried soil is transferred from a metal cup holding 2.5 grams to an Erlenmeyer flask of 125-ml. capacity. Thirty ml. of a neutral solution of ammonium acetate are added to the soil from a dispensing burette. The soil and reagent are given approximately 100 swirls in about one-half minute, and immediately poured into a previously prepared dry filter and collected in a 50-ml. beaker. One ml. of



Rapid Chemical Method for the determination of calcium in soil.

the filtrate is transferred to a short, flat-bottom comparison vial and is made up to 2 ml. with the calcium solvent. One ml. of calcium precipitant consisting of oxalic acid dissolved in dilute acetic acid is added from a calibrated dropper. The

tube containing the mixture is closed with the thumb and given 30 rapid shakes. The solution is allowed to stand for one-half minute and is then placed on the calcium illuminator. This instrument is similar to the potash illuminator in design, but contains its own standardized card of 3 sets of lines. The calcium content varies directly as the turbidity of the solution and this is measured in terms of the visibility or non-visibility of the lines on the calcium card. The readings are referred to a table accompanying the assembly which gives the calcium content in terms of per cent CaO and pounds CaO per acre-foot.

For each soil sample determinations are made with different aliquots of the filtrate until consecutive readings of both 2 and 3 are obtained. By referring to the table the lower figure represented by either one of these readings is recorded as the final result. The consecutive readings of 2 and 3 are required to select an accurate range of the calcium concentration, for unlike the rapid method for potash, usually 2 or more readings of 2 can be obtained. The lower figure indicated by the consecutive readings in the table is reported because the chart (special for calcium) has been calibrated to give readings of 2 and 3 at two definite minimum concentrations of calcium.

#### *The Determination of Total Calcium in Filter Cake:*

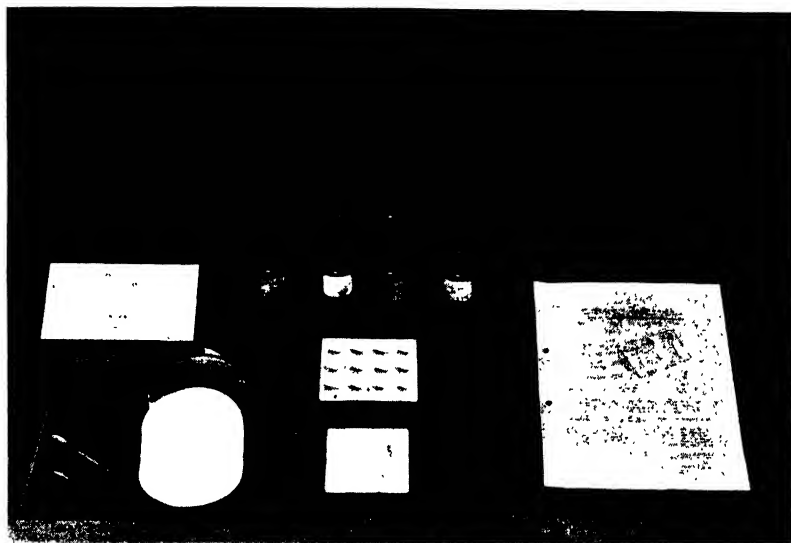
A weighed amount of filter cake is extracted with a measured volume of a one-half normal solution of hydrochloric acid. To 5 ml. of the extract is added an equal volume of 0.48 normal solution of sodium hydroxide. Successively increasing aliquots of 0.1 ml. to 1.5 ml. of the mixed solution are transferred to short shell vials. Four such aliquots are taken for each reading. The contents of the vials are made to 2 ml. with a solution of neutral normal ammonium acetate. A precipitate of calcium oxalate is formed by the addition of 1 ml. of a reagent consisting of oxalic acid in dilute acetic acid solution. The vial is closed with the thumb. It is then given 30 vigorous shakes in 7 seconds. The turbidity produced as a result of this treatment is viewed against a chart of ruled, parallel green lines in groups of increasing intensity before a background of diffused light in a specially prepared illuminating device. The illuminator slide is placed so that the slots open on the No. 3 lines of this chart. The extent of turbidity produced in this analysis is measured by the masking effect of the oxalate suspension upon the chart grouping of the darkest green lines. Three tubes are placed in the slide each time. The tube through which the lines are clearly visible is removed and the fourth tube is placed on the slide. Notation is made of that aliquot which produces a turbidity such that upon sighting through the column of precipitate the heavy ruled or No. 3 lines are just barely visible. The reading for this aliquot is "3." Then the next larger aliquot in which the lines are not visible is noted, a "4" reading. The readings are referred to a table and the results for the 2 aliquots are obtained. The final value representing the analysis of the sample is the average of the values for the 2 selected readings. The result is expressed as per cent CaO and pounds CaO per ton of filter cake.

#### *The Colorimetric Determination of Soil Reaction (pH):*

The procedure for the rapid colorimetric determination of soil reaction is sim-

ple in principle. It consists essentially in passing a suitable organic pH indicator through a small sample of soil and noting the color change of the indicator brought about by the free hydrogen or hydroxyl ions present in the soil. The final color thus obtained is compared with a color chart of that particular indicator and when a match is obtained, the pH reading is taken directly from the chart.

Preliminary tests run with an artists' porcelain spot plate serve to determine the proper indicator to be used for any particular soil and at the same time give the approximate pH readings.



Rapid Chemical Method for the colorimetric determination of soil reaction (pH).

Having determined the correct indicator for the soil under analysis, the test is repeated, using the LaMotte-Morgan pH block with which the final and more accurate result is obtained. A small sample of soil is placed in the compartment above the perforated partition and the indicator added drop by drop until the soil is saturated. Two to 3 drops more are added, and by a very light application of the sharp end of a glass rod against the perforations of the partition on the side opposite the soil, the solution is drawn out by capillary action. This solution is then drawn down the channel into the lower depression by using the large end of the rod. The color of the liquid thus obtained is immediately matched with the color chart of the particular indicator used and the pH reading taken directly from the chart.

#### THE REAGENTS USED IN R.C.M. WORK

The numbering of reagents in the analytical directions which follow may appear to be erratic. The reasons for this are that some of the older reagents have been discontinued while others have been given numbers by adding a cipher (0) or a ten (10) to an older reagent designation in order to associate a newer modification with a much used and earlier standard solution.

### *A Word Regarding Reagents:*

We have found by experience that the only possible manner of insuring accurate and reliable R.C.M. analyses is by centralizing the preparation of the various reagents for the whole Hawaiian enterprise in the hands of trained chemists. Sporadic and perfectly good-intentioned excursions into this field by a few untrained workers have resulted in the loss of otherwise good laboratory effort in worthless analyses. Losses of money and time and delays to execution of work have also resulted from the employment of unfit or faulty reagents. The Experiment Station assumes the responsibility of maintaining the highest possible quality in the reagents supplied from the Honolulu laboratories. The inspection service by the Experiment Station is designed, among other objectives, to maintain the high quality of reagents under laboratory usage by the plantation staffs.

#### *Reagent No. 1, $K_2O$ :*

An aqueous solution of sodium acetate, C. P., 500 grams in 8000 ml. of distilled water. To 10,500 ml. of this solution are added 4500 ml. of C. P. nitric acid in 1-1 solution.

#### *Reagent No. 2, $K_2O$ :*

A solution of C. P. cobaltous nitrate, 318 grams and C. P. sodium nitrite, 1200 grams in distilled water, 4000 ml. containing 100 ml. of C. P. acetic acid. This reagent must remain for a time in storage in the dark, later is filtered, tested and shipped in small, amber glass-stoppered containers. It decomposes rather readily in the tropics and requires occasional inspection.

#### *Reagent No. 3, $K_2O$ :*

Redistilled ethyl alcohol, exactly 95 per cent, containing the denaturation pyridine and xylol.

#### *Reagent No. 4, $P_2O_5$ :*

A solution of 100 grams C. P. ammonium molybdate and 850 ml. of distilled water—stored, aged and filtered. Seventeen hundred ml. C. P. concentrated hydrochloric acid (free from arsenic and phosphates) is added to 700 ml. distilled water. The above solutions are combined and then stored in large “non-sol” glass containers. This is the concentrated stock solution. To prepare the reagent, 120 parts of stock solution are diluted to 1000 parts with distilled water. The finished reagent is stored and shipped in paraffined glass containers.

#### *Reagent No. 5, $N$ :*

This is an approximately 0.3 normal solution of C. P. potassium sulfate.

#### *Reagent No. 6, $N$ :*

A solution of mercuric chloride is saturated by boiling with an excess of the salt. A solution of potassium iodide is prepared by dissolving 61.75 grams of the C. P. chemical in 250 ml. of distilled water.

To the potassium iodide solution an amount of the cold mercury solution is added, just sufficient to make the color a permanent bright red. The red precipitate is dissolved by adding exactly 0.75 gram of potassium iodide. Next add a solution of 150 grams C. P. potassium hydrate dissolved in 250 ml. of distilled water. Add distilled water to 1000 ml. Allow to settle clear, age for a time, test and bottle.

It is best to make up a large amount of Nessler solution. If, by its use, the ammonia standards do not match the artificial ones prepared, a little more mercuric chloride to increase sensitiveness, or potassium iodide to decrease it, will bring the Nessler's solution to the point where, if just 1 ml. is used, the regular ammonia standards will exactly match the artificial ones. The artificial standards may then be employed for the ammonia readings until the Nessler solution tested is entirely used. Of course, each new lot of Nessler solution should be compared to see that it has the proper degree of sensitiveness to match the standards.

*Reagent No. 6A, N:*

Dissolve 500 grams sodium-potassium tartrate, C. P. and free from nitrogen in 1000 ml. of ammonia-free distilled water. Add 5 ml. Reagent 6, N, for each 50 grams of salt dissolved. Stir and let stand overnight. Add an additional few drops of Reagent 6, N, to detect any unprecipitated ammonium salt. If found, repeat addition of Reagent 6, N, let stand as before, and continue in this manner until no further precipitate is formed. Filter and age the solution by storing for a while. Dispense in rubber-stoppered bottles.

*Reagent No. 7, N:*

Dissolve 200 grams pure white phenol in 1200 ml. of N-free sulfuric acid. Add 600 ml. fuming sulfuric acid (15 per cent  $\text{SO}_3$ ) and stir well. Heat for 2 hours at  $100^\circ \text{C}$ . Store in clean glass containers. Dispense in glass-stoppered bottles.

*Reagent No. 8, N:*

Distilled water free of ammonia.

*Reagent No. 9, N:*

C. P. concentrated ammonium hydroxide.

*Reagent No. 10,  $\text{K}_2\text{O}$ :*

A 10 per cent solution in distilled water of Reagent No. 1,  $\text{K}_2\text{O}$ .

*Reagent No. 13,  $\text{CaO}$ :*

Dissolve 1232.96 grams C. P. ammonium acetate in 16,000 ml. distilled water. Age for a few months before adjusting pH to neutrality.

*Reagent No. 14,  $\text{CaO}$ :*

Dissolve 32 grams C. P. oxalic acid in 1000 ml. of a 36 per cent acetic acid solution.

*Reagent No. 15, Total N:*

Concentrated C. P. sulfuric acid free from nitrogen, containing 0.25 gram selenious oxide per 100 ml.

*Reagent No. 16, Total N:*

A solution consisting of 40 ml. normal sulfuric acid diluted to 1500 ml. with distilled water.

*Reagent No. 17, Total N:*

A solution of sodium hydroxide, 275 grams dissolved in 750 ml. of water, boiled for 5 minutes. Cool and store.

*Reagent No. 18, Total N:*

Redistilled water by special treatment to render it free from ammonia and other impurities.

*Reagent No. 19, CaO:*

A 0.48-N solution of C. P. sodium hydroxide.

*Reagent No. 40,  $P_2O_5$ :*

A solution of 133 grams C. P. ammonium molybdate and 850 ml. distilled water is aged and filtered. A mixture of 2267 ml. C. P. concentrated hydrochloric acid (arsenic and phosphate free) and 133 ml. of distilled water is prepared. These solutions are combined and stored in large "non-sol" glass containers. This is the stock solution. To prepare the reagent, 120 parts of the stock solution are diluted to 1000 parts with distilled water. The finished reagent is stored and shipped in paraffine-lined glass containers.

*Series 100 Phosphate Fixation Solution:*

One hundred parts per million  $P_2O_5$  from diammonium phosphate, plus 160 grams potassium sulfate per 16,000 ml. of reagent.

*Series 500 Phosphate Fixation Solution:*

Five hundred parts per million  $P_2O_5$  from diammonium phosphate, plus 240 grams potassium sulfate per 16,000 ml. of reagent.

*Series 1000 Phosphate Fixation Solution:*

One thousand parts per million  $P_2O_5$  from diammonium phosphate, plus 480 grams potassium sulfate per 16,000 ml. of reagent.

*Stannous Chloride Solution:*

A  $2\frac{1}{2}$  per cent solution of C. P. stannous chloride dissolved in 1:10 hydrochloric acid. Observe usual precautions of maintaining tin in stannous form.

*Ammonium Molybdate Solution:*

Dissolve 25 grams of ammonium molybdate in 200 ml. distilled water heated to 60° C. Filter. Dilute 280 ml. of arsenic and phosphate-free, concentrated sulfuric acid (36 N) to 800 ml. with distilled water. When cool, combine the 2 solutions with care. When again cool, dilute to 1000 ml. This is a stock solution. It should be aged in the dark. The reagent is prepared by diluting the stock solution with equal parts of distilled water.

*Bromthymol Blue pH Indicator:*

Pipette 40 ml. of 1 per cent bromthymol blue stock solution into a 1000-ml. volumetric flask and ~~make up~~ almost to mark, using CO<sub>2</sub>-free distilled water. Mix thoroughly and remove a drop on an artists' spot plate. Compare color with bromthymol blue color chart. Adjust indicator to pH 6.8, using very dilute NaOH solution (about 0.05 N) if indicator is too acid, or dilute HNO<sub>3</sub> solution (about 0.05 N) if it is too alkaline, mixing thoroughly and sampling after each addition.

*Bromcresol Green pH Indicator:*

Prepare in a similar manner, using 40 ml. of 1 per cent bromcresol green stock per liter and adjusting pH to 4.6.

*Chlorphenol Red pH Indicator:*

Use 40 ml. of 1 per cent chlorphenol red stock per liter and adjust pH to 5.8.

*Phenol Red pH Indicator:*

Use 20 ml. of 1 per cent phenol red stock per liter and adjust pH to 7.4.

*Hydrochloric Acid:*

Chemically pure concentrated acid (free from arsenic and phosphates).

*Nitric Acid:*

Chemically pure concentrated acid (free from arsenic and phosphates).

*N/2 Hydrochloric Acid Solution:*

Forty ml. concentrated hydrochloric acid diluted to 1 liter with distilled water.

*Potassium Sulfate:*

Chemically pure powder, low in nitrogen content.

## R.C.M. COLOR STANDARDS

The preparation of these standards from organic dyes is an elaborate and involved process. Details of preparation are to follow but it may be stated that the various standards are being replaced as rapidly as research makes it possible by solutions of inorganic ions in media of approximately identical physical characteristics to the test solutions formed in the colorimetric analyses.



*Soil Phosphate:*

The stock reagents are prepared as follows:

Reagent "A"—Brilliant Wool Blue G. Extra, Schultz, No. 565.

Dissolve 0.5 gram in 1000 ml. distilled water.

Reagent "B"—Metanil Yellow, Schultz No. 134.

Dissolve 0.25 gram in 1000 ml. distilled water.

Reagent "C"—Erythrosine, Schultz No. 592.

Dissolve 0.1 gram in 500 ml. distilled water.

Reagent "D"—Brilliant Milling Green B, Schultz No. 503.

Dissolve 0.5 gram in 1000 ml. distilled water.

To prepare the color standards, mix the following portions in the order given, mixing and washing down the side of the flask with sufficient distilled water after each addition. Start with about 150 ml. of distilled water in each flask. The stock reagents and standards should be stored in a dark place away from sunlight.

P <sub>2</sub> O <sub>5</sub> Group	ml. of "A"	ml. of "B"	ml. of "C"	ml. of "D"	ml. Total Volume
Standard Series "X"					
High .....	33	11	0	0	250
Medium .....	25	10	0	0	250
Doubtful .....	14	6	0	0	250
Low .....	Water	.	.	.	250
Standard Series "Y"					
High .....	30	9	0.5	2.5	250
Medium .....	17	4.5	1.0	0.2	250
Doubtful .....	6	2.0	0.5	2.0	250
Low .....	0	2.5	0	0	250

*High-Register Soil Phosphate:*

The stock reagents are prepared from:

Reagent 2A—Brilliant Wool Blue G. Extra, Schultz No. 565.

Dissolve 1.0 gram in 1000 ml. distilled water.

Reagents B, C and D—These reagents are prepared in a similar manner to those for the soil phosphate standards.

In preparing the standards, the following portions are mixed in the order given with subsequent additions of water:

No.	Per Cent P <sub>2</sub> O <sub>5</sub>	Lbs./a-ft.	Equiv. to No. ml. of 0.1 mg. P <sub>2</sub> O <sub>5</sub> /ml. made up to 100 ml.	ml. "2A"	ml. "B"	ml. "C"	ml. "D"	ml. Total Volume
I	.004	100	8	15	9	0.5	2.5	250
II	.006	150	12	21	10	0.5	5.0	250
III	.008	200	16	28	11	0.5	7.0	250
IV	.010	250	20	35	13	0.5	10.0	250
V	.012	300	24	45	15	0.5	18.0	250
VI	.014	350	28	55	17	1.0	25.0	250
VII	.016	400	32	65	20	1.0	35.0	250

*Phosphate in Cane Juice Color Standards:*

The stock reagents (reagents A, B, C and D) are identical to those used for soil phosphate standards. The color standards are prepared as follows:

No.	Equiv. to No. ml. of 0.1 mg. P <sub>2</sub> O <sub>5</sub> /ml. made up to 100 ml.	ml. "A"	ml. "B"	ml. "C"	ml. "D"	ml. Total Volume
1	4	13	4.3	0.5	0.3	250
2	6	18	5.0	0.5	1.0	250
3	8	30	9.0	0.5	2.5	250
4	10	35	9.0	0.5	4.0	250
5	12	42	10.0	0.5	5.0	250
6	14	48	10.0	0.5	6.0	250
7	16	55	11.0	0.5	7.0	250
8	18	62	12.0	0.5	8.0	250

*Soil Phosphate Fixation Color Standards:*

The stock solutions are made up as follows:

A. Methylene Blue—Merck Reagent Grade: Transfer 0.50 gm. methylene blue to a 500-ml. volumetric flask; add sufficient distilled water to dissolve the dye. Make up to volume, stopper and mix solutions thoroughly. Use this as stock solution "A."

B. Aniline Yellow—Eimer & Amend Tartrazine: Transfer 0.25 gm. aniline yellow to a 250-ml. volumetric flask. Dissolve the dye with sufficient water and make up to mark. Stopper and mix solution thoroughly. Filter. Use filtrate as stock solution "B."

C. Fuchsin: Transfer 0.10 gm. fuchsin to a 500-ml. volumetric flask. Add sufficient hot water (below boiling point) to dissolve the crystals. Cool to room temperature and make up to mark. Stopper and mix solution thoroughly. Use this as stock solution "C."

To 250-ml. volumetric flasks pipette the given amounts of reagents "A," "B" and "C" as specified in the table, subject to the following instructions:

1. To each flask add 175 ml. distilled water.
2. Pipette accurately the required amount of methylene blue stock solution "A" to each flask. Wash down reagent adhering to neck of flask. Mix the liquid thoroughly.
3. Add by pipetting the required amount of aniline yellow, stock solution "B" and repeat the process of washing and mixing.
4. Add by pipetting the required amount of fuchsin, stock solution "C" and repeat the washing and mixing process.
5. Make up to mark with distilled water. Stopper and mix the solution thoroughly.
6. Remove the stopper, add 1 drop thymol-menthol solution to each 250-ml. color standard as prepared above. Stopper and mix thoroughly: The color standards are now ready to be transferred to vials. (Always mix contents thoroughly)

before transferring.) To prevent contamination by organic substances present in corks, the vials should be rubber stoppered.

7. The above order of adding reagents should be strictly adhered to in order to insure complete blending of colors and to prevent precipitation. The oily bactericide should be added after the solution has been made up to volume and mixed. It has been found that the oil will absorb a very small amount of red coloring matter if it is allowed to come into contact with the concentrated reagents.

Index No.	ml. Methylene Blue "A"	ml. Aniline Yellow "B"	ml. Fuchsin "C"	ml. Total Volume
0	25.0	4.00	4.00	250
10	19.50	3.00	3.00	250
20	14.50	2.00	3.00	250
30	10.50	1.60	3.40	250
40	7.50	1.00	2.60	250
50	5.00	0.70	2.50	250
60	3.50	0.50	2.10	250
70	2.20	0.40	1.60	250
80	2.50	0.50	1.50	500
90	1.60	0.30	0.80	500

#### *Soil Nitrogen Color Standards:*

The stock solutions to be used in the preparation of these color standards are made up as follows:

Solution III.—Dissolve 0.1 gm. Erythrosine 592 in a liter of distilled water.

Solution IV.—Dissolve 0.5 gm. Metanil Yellow in a liter of distilled water.

The following proportions of Solutions III and IV are used to prepare the ammoniacal nitrogen color standards:

(1 cup, 10 gm. soil—50 ml. N—5 ml. filtrate)					
No.	Per Cent N (NH <sub>4</sub> )	Lbs. N/ a-ft.	0.5 gm./L ml. M. Yel.	0.1 gm./L ml. Eryth. (red)	ml. Total Volume
1	.0002	5	1.00	0.50	250
2	.0006	15	2.20	0.90	250
3	.0010	25	3.60	1.70	250
4	.0014	35	4.70	2.70	250
5	.0018	45	6.50	4.00	250
6	.0024	60	9.00	6.00	250
7	.0030	75	12.00	9.00	250
8	.0040	100	18.00	15.00	250

The nitrate nitrogen color standards consist of mixtures of aqueous solutions of potassium chromate and potassium dichromate. Each solution contains 5 grams of the salt per liter. The exact proportion in which the solutions must be mixed to give each standard is presented in the following table:—

Solution I—K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 5 gm./L.

Solution II—K<sub>2</sub>CrO<sub>4</sub> 5 gm./L.

Standard equiv. to Lbs. $\text{NO}_3 \text{ N/a-ft.}$	ml. Soln. I per 250 ml.	ml. Soln. II per 250 ml.
5	3.25	0
13	7.50	0
25	7.50	7.50
38	8.75	13.75
50	11.25	21.25
75	13.75	31.25
100	16.25	43.75
125	20.00	80.00

Solutions I and II in the proportions given above are added to a 250-ml. volumetric flask from burettes and made up to volume with distilled water. The solution is mixed and transferred into vials which are then stoppered and sealed.

#### DETAILED R.C.M. PROCEDURES

Detailed directions for analyses of soil, plant material, crusher juice, mill by-products, and irrigation waters follow.

#### RAPID ESTIMATION OF AVAILABLE NITROGEN IN SOILS

##### *Equipment Required*

- 6 Flasks, Erlenmeyer, 125-ml. cap.
- 12 Beakers, Pyrex, 100-ml. cap.
- 1 Box No. 12 Whatman folded filter paper, 15 cm.
- 1 Funnel support.
- 6 Funnels, 65 mm.
- 1 Pipette, Exax, volumetric, 25-ml. cap.
- 1 Pipette, Exax, volumetric, 5-ml. cap.
- 1 Metal soil cup, 10-gram cap.
- 1 Spatula, stainless steel, 4-inch blade.
- 1 Support, iron, 6 in. by 9 in.
- 1 Clamp, burette, castaloy, large, with rubber-covered jaws.
- 1 Burette, dispensing, 250-ml. cap.
- 1 Cover for 250-ml. dispensing burette.
- 10 Vials, shell, tall form.
- 10 Vials, tall form (reserved for ammoniacal and Total N).
- 1 Pipette, special, to deliver Reagent 7, N, 2-ml. cap.
- 1 Pipette, special, to deliver Reagent 6, N, 1-ml. cap.
- 10 Rubber stoppers, No. 00.
- 1 Electric hot plate.
- 1 Set ammonia color standards
- 1 Set nitrate color standards
- } in box with 2 pcs. of black sateen.
- 6 Glass rods, 4 inches long.
- 1 Phosphate illuminator.
- 1 Bottle, dropping, pipette stopper with nipple, 30-ml. cap. for Reagent 6A, N.
- 1 Cylinder, graduated, 25-ml. cap.

- 1 Pipette, Mohr, 5-ml. cap.
- 1 Pipette, Mohr, 10-ml. cap.
- 1 gal. Reagent 5, N.
- $\frac{1}{4}$  pt. Reagent 6, N.
- $\frac{1}{4}$  pt. Reagent 6A, N.
- $\frac{1}{4}$  pt. Reagent 7, N in g. s. b.
- 1 qt. Reagent 8, N.
- 1 qt. Reagent 9, N.
- 1 qt. Reagent 18, Total N.

#### *Additional Equipment Recommended*

- 2 Burettes, dispensing, 250-ml. cap. (with covers) instead of 1 cylinder, graduated, 25-ml. cap. (with proper supports).

#### *Equipment Required For Soil Nitrogen Determinations Not Included in Potash or Phosphate Ensembles*

- 10 Vials, shell, tall form (reserved for ammoniacal and Total N).
- 12 Beakers, Pyrex, 100-ml. cap.
- 1 Box No. 12 Whatman folded filter paper, 15 cm.
- 1 Pipette, Exax, volumetric, 25-ml. cap.
- 1 Pipette, Exax, volumetric, 5-ml. cap.
- 1 Pipette, special, to deliver Reagent 7, N, 2-ml. cap.
- 1 Pipette, special, to deliver Reagent 6, N, 1-ml. cap.
- 10 Rubber stoppers, No. 00.
- 1 Set ammonia color standards
- 1 Set nitrate color standards } in box with 2 pcs. of black sateen.
- 6 Glass rods, 4 inches long.
- 1 Bottle, dropping, 30-ml. cap., for Reagent 6A, N.
- 1 Cylinder, graduated, 25-ml. cap.
- 1 Pipette, Mohr, 5-ml. cap.
- 1 Pipette, Mohr, 10-ml. cap.
- 1 gal. Reagent 5, N.
- $\frac{1}{4}$  pt. Reagent 6, N.
- $\frac{1}{4}$  pt. Reagent 6A, N.
- $\frac{1}{4}$  pt. Reagent 7, N g. s. b.
- 1 qt. Reagent 8, N.
- 1 qt. Reagent 9, N.
- 1 qt. Reagent 18, Total N.

#### *Extraction*

1. Fill a 10-gram metal cup with prepared soil and level off with a stainless steel spatula. Transfer to a 125-ml. Erlenmeyer flask.
2. Add 50 ml. of Reagent 5, N, from a 250-ml. dispensing burette.
3. Shake 1 minute.
4. Filter through No. 12 Whatman folded filter paper, 15 cm., into a 100-ml. beaker.

### *Determination of Ammoniacal Nitrogen*

1. Pipette 5 ml. of the filtrate (step No. 4 above) into a tall form, shell vial.

*Precautions:* Reserve a set of these vials exclusively for ammoniacal and total nitrogen determinations. Do not use vials employed in other studies. Do not carry on ammoniacal nitrogen determinations until all bottles containing Reagent 9, N, are closed and all vessels containing this reagent have been emptied and washed.

2. Add 4 drops Reagent 6A, N.
3. Add 1 ml. Reagent 6, N, with the special pipette.
4. Stopper vial with a No. 00 rubber stopper and let stand for 1 minute.
5. Shake vigorously to disperse all the precipitate that may be formed upon the addition of Reagent 6, N, to the filtrate.
6. Transfer the test vial to the rack of ammonia color standards, place on a phosphate illuminator and make comparisons.

7. Results are directly indicated on the standard color vials and are expressed as per cent N (ammoniacal) and pounds N per acre-foot of 2.5 million pounds soil.

8. When the amount of ammoniacal nitrogen exceeds 100 pounds per acre-foot, proceed as follows:

9. Using a 5-ml. Mohr pipette, transfer a suitable aliquot ( $2\frac{1}{2}$ , 2, 1,  $\frac{1}{2}$  ml., etc.) of the original 10-gram-50-ml. extract into a tall vial.

10. Dilute to 5 ml. with Reagent 18, Total N by means of a 10-ml. Mohr pipette.

*Precaution:* In making dilutions with Reagent 18, Total N, pour out enough into a beaker and use the solution in the beaker. After completion of a set of analyses, discard the remaining solution.

11. Proceed with steps Nos. 2 to 6 of ammoniacal nitrogen.

12. Multiply the results by a factor obtained by dividing 5 by the aliquot of filtrate taken. For example:

Aliquot taken =  $2\frac{1}{2}$  ml. — Multiply result by  $5/2\frac{1}{2}$  or 2.

Aliquot taken = 2 ml. — Multiply result by  $5/2$  or  $2\frac{1}{2}$ .

Aliquot taken =  $\frac{1}{2}$  ml. — Multiply result by  $5/\frac{1}{2}$  or 10.

### *Determination of Nitrate Nitrogen*

1. Pipette 25 ml. of filtrate (step No. 4, extraction) into a clean 100-ml. beaker.
2. Evaporate on a hot plate nearly to dryness at low heat. (The residue may be dry but should *not* be allowed to bake.)

*Precaution:* It is not desirable to proceed beyond step No. 5, below, while other nitrogen studies (ammoniacal and total nitrogen) are in progress. It will be convenient to postpone steps Nos. 3 to 10 until other nitrogen studies have been terminated.

3. Let cool 1 minute and add 2 ml. of Reagent 7, N, using the special pipette.

4. Mix well by means of the glass rod, breaking up the residue to insure its intimate contact with the reagent. Let stand 5 minutes.

5. Add, either from a graduated cylinder or a 250-ml. dispensing burette, 10 ml. of Reagent 8, N, and stir well to effect solution.

6. Using the same graduate as in step No. 5, or a separate 250-ml. dispensing

burette, add 15 ml. of Reagent 9, N, agitating vigorously the contents of the beaker at the same time.

*Precaution:* Keep bottles of Reagent 9, N, in a separate place away from other reagents. When through dispensing this reagent, empty graduates or burettes and wash immediately. Empty and wash other vessels to which Reagent 9, N, has been added as soon as convenient.

7. Allow any insoluble residue to settle and decant the clear portion of the liquid into a tall form vial.

8. Transfer the test vial to the rack of nitrate standards placed on the phosphate illuminator and make comparisons.

9. Read off directly from the standard vial the concentration of nitrogen expressed as per cent N (nitrate) and pounds of N per acre-foot.

10. When the amount of nitrate nitrogen in the soil exceeds 125 pounds per acre-foot, proceed as follows:

11. Using a 10-ml. Mohr pipette, transfer a suitable aliquot (4 ml., 2 ml., 1 ml., etc.) of the solution in which the nitrate color has been developed (step No. 6 above) into a tall vial. Wash the pipette.

12. Add with the 10-ml. Mohr pipette amounts of Reagent 8, N, indicated in the tabulation following step No. 14, below.

*Precaution:* Pour out enough Reagent 8, N, into a beaker and use the solution in the beaker, discarding the remaining solution after completion of a set of analyses.

13. Cover vial with index finger and mix solution completely by inverting several times.

14. Compare with standards and multiply results by a factor obtained by dividing the total volume by the aliquot taken:

Aliquot Taken	Reagent 8, N, Added	Total Volume	Factor
4	4	8	2
2	4	6	3
2	6	8	4
1	4	5	5

#### *Precautions*

1. Keep Reagent 9, N, when not in actual use, in a separate place away from other reagents. Do not use when determination of nitrogen in forms other than the nitrate are in progress.

2. Color standards should be kept in closed boxes when not in actual use.

3. Reagents 5, N, and 7, N, should not be put in the same cupboard with or left adjacent to concentrated nitric acid or Reagent 2,  $K_2O$ .

#### RAPID ESTIMATION OF TOTAL NITROGEN IN FILTER CAKE

(Sample to be weighed out on an analytical balance)

1. Obtain a representative sample of the filter cake.

2. Break up large lumps by crumbling with hand. Mix material thoroughly. The sample is now ready for analysis without drying.

3. Weigh 2.5 gm. of the sample and transfer to Kjeldahl flask, R. C. M. Drop in 4 glass beads. Add 1 to 2 grams of special potassium sulfate. Introduce 20 ml. Reagent 15, Total N, with graduated cylinder. Let stand 5 minutes.

4. Place Kjeldahl flask on heater to digest. Insert gas absorption Hengar tube into neck of flask.

5. Digest for *one* hour.

6. Cool to room temperature. Add 100 ml. Reagent 8, N, from graduated cylinder, washing down neck of Kjeldahl flask. Cool slightly. Transfer by pouring into 250-ml. volumetric flask through a 65-mm. funnel. Rinse Kjeldahl flask several times with Reagent 8, N, transferring the rinsings to the 250-ml. volumetric flask. When the solution in the volumetric flask is cool, make volume up to 250-ml. mark with Reagent 8, N. Stopper and mix thoroughly.

7. Pipette 10 ml. of the solution from the volumetric flask into a clean Kjeldahl flask. Drop in 2 glass beads. Add 100 ml. Reagent 8, N, and 20 ml. Reagent 17, Total N. Connect flask with distillation apparatus. Shake flask slightly. Immediately place Kjeldahl flask on heater and introduce distillation outlet into the calibrated test tube to which has been added 15 ml. Reagent 16, Total N. The test tube is immersed in cold water in a 500-ml. Erlenmeyer flask which acts as a condenser.

8. Remove test tube when nearly 50 ml. have been distilled over. Cool distillate to room temperature. (Tube is removed before heat is shut off from the distilling flask.)

9. Make volume to exactly 50 ml. with Reagent 8, N. Mix thoroughly.

10. Pipette 5 ml. into comparison vial. Add 1 ml. Reagent 6, N; let stand 3 minutes. Stopper and mix well. (The original distillate may be used or dilutions of the original may be made. However, for both original distillate or diluted aliquots, 5-ml. portions are used for color development. The dilution should be thoroughly mixed before the 5-ml. portions are withdrawn for color development with Reagent 6, N.)

11. Compare with *Soil Ammoniacal Standards*, using the illuminator. Refer to table for results.

#### Data for Total N in Filter Cake

2.5-gm. Sample — 250 ml. Digested Soln.

10-ml. aliquot used in distillation, equivalent to 0.1-gm. sample.

50-ml. Distillate Obtained.

#### PER CENT TOTAL N IN FILTER CAKE

Distillate diluted as indicated below:\*

Reading Tube No.	5 ml. Orig. Distillate		5 ml. Orig. Dist. 5 ml. Reag. 8		5 ml. Orig. Dist. 15 ml. Reag. 8		5 ml. Orig. Dist. 35 ml. Reag. 8	
	Per	lb/ton	Per	lb/ton	Per	lb/ton	Per	lb/ton
	Cent N		Cent N		Cent N		Cent N	
1	..	..	..	..	..	..	..	..
2	.06	1.2	.12	2.4	..	..	..	..
3	.10	2.0	.20	4.0	.40	8.0	.80	16.0
4	.14	2.8	.28	5.6	.56	11.2	1.12	22.4
5	.18	3.6	.36	7.2	.72	14.4	1.44	28.8
6	.24	4.8	.48	9.6	.96	19.2	1.92	39.4
7	.30	6.0	.60	12.0	1.20	24.0	2.40	48.0
8	.40	8.0	.80	16.0	1.60	32.0	3.20	64.0

\* Use 5 ml. of the final solution, diluted in proportions as indicated, for color development.



## RAPID ESTIMATION OF TOTAL NITROGEN IN SOIL

1. Quarter prepared soil until about one-half pound remains.
2. Grind this material to a fine powder with a mortar and pestle. Mix thoroughly.
3. Weigh 2.5 grams of the sample and transfer to a 300-ml. Kjeldahl flask. Drop 4 glass beads into the flask. Add 1 to 2 grams of potassium sulfate. Introduce 20 ml. Reagent 15, Total N, from a graduated cylinder.
4. Let stand for about 5 minutes. Fit a Hengar tube into the neck of the Kjeldahl flask and place it on the electric heater to digest.
5. Digest for 1 hour. Using the wooden clamp, remove flask to the box. Cool to room temperature.
6. Add 100 ml. Reagent 18, Total N, from a graduated cylinder, washing down the neck of the flask. Cool. Carefully decant off the supernatant liquid into a 250-ml. volumetric flask through a 65-mm. funnel. Rinse the Kjeldahl flask several times with small portions of Reagent 18, Total N, letting sediment settle and decanting off supernatant liquid each time into the volumetric flask. When the solution in the volumetric flask is cool, make volume up to 250-ml. mark with Reagent 18, Total N. Stopper and mix thoroughly.
7. To a large, calibrated test tube add 15 ml. of Reagent 16, Total N. Immerse it in cold water in a 500-ml. Erlenmeyer flask which acts as a condenser. Set the condenser on an iron support shelf and arrange this unit adjacent to heater unit for the distillation.
8. Pipette 10 ml. of the solution from the volumetric flask into a clean Kjeldahl flask. Drop in 2 glass beads. Add 100 ml. Reagent 18, Total N, and a pinch of granulated zinc (20 mesh). Turn on heater current. Add 20 ml. Reagent 17, Total N, from a special dispensing burette, introducing it carefully down the inner side of the flask. Immediately attach the connecting bulb assembly, stoppering "trap end" tightly to the Kjeldahl flask. The outlet tube is put into the test tube with the tip momentarily withheld above the level of Reagent 16, Total N, while the contents of the Kjeldahl flask is agitated thoroughly. After mixing, the tip of the distillation outlet tube is immersed in the 15 ml. of Reagent 16, Total N. Immediately place the Kjeldahl flask on the heater.
9. Distillation is continued until the distillate reaches the level marked on the test tube, when the condensing flask and tube are immediately removed from the distillation outlet. The heater may then be turned off and the connecting bulk assembly removed. The test tube is removed from the Erlenmeyer flask and placed on the tube rack to cool.
10. Make volume up to exactly 50 ml. with Reagent 18, Total N. Insert a clean rubber stopper and mix thoroughly.
11. Pipette 5 ml. into a comparison vial. Add 1 ml. Reagent 6, N; let stand 3 minutes. Stopper and mix well. (The original distillate may be used or dilutions of the original may be made. However, for both original distillate or diluted aliquots, 5-ml. portions are used for color development. The dilution should be thoroughly mixed before the 5-ml. portions are withdrawn for color development with Reagent 6, N.)

12. Compare with *Soil Ammoniacal Standards*, using the illuminator. Refer to table for results.

#### DATA FOR TOTAL N IN SOILS

2.5-gm. sample -- 250 ml. digested soln.  
10-ml. aliquot used in distillation equiv. to 0.1-gm. sample.  
50 ml. distillate obtained.

Reading Tube No.	5 ml. Original Distillate		5 ml. Original Dist. 5 ml. Reag. 18, T N		5 ml. Original Dist. 15 ml. Reag. 18, T N	
	Per Cent N	lb/a-ft.	Per Cent N	lb/a-ft.	Per Cent N	lb/a-ft.
2	.06	1,500	.12	3,000	..	.....
3	.10	2,500	.20	5,000	.40	10,000
4	.14	3,500	.28	7,000	.56	14,000
5	.18	4,500	.36	9,000	.72	18,000
6	.24	6,000	.48	12,000	.96	24,000
7	.30	7,500	.60	15,000	1.20	30,000
8	.40	10,000	.80	20,000	1.60	40,000

#### RAPID DETERMINATION OF NITROGEN IN MILL WATER AND IN WATER CONTAINING ORGANIC MATTER

Use the same equipment as is required in making determinations of total nitrogen in cane juice and available nitrogen in soil.

##### *General Procedure:*

Collect 1 gallon of water as representative of the sample to be analyzed. Thoroughly mix in bottle. Filter about 250 ml. through a Whatman No. 12 15-cm. filter paper. Nitrogen is determined by two procedures: (I) Organic plus ammoniacal nitrogen determination and (II) nitrate nitrogen analysis.

##### *I. Determination of Organic Plus Ammoniacal Nitrogen:*

1. Measure 100 ml. of the filtered sample and transfer to a Kjeldahl flask. Drop 2 glass beads into flask. Add approximately .3 gm. potassium sulfate powder (about 1/3 spoonful—small horn spoon supplied in N-in-cane-juice assembly). Introduce 4 ml. Reagent 15, Total Nitrogen, by special pipette.

2. Heater is turned on and the Kjeldahl flask is placed on it.

3. Boil until water is evaporated and white fumes are formed in neck of flask. Attach Hengar tube and continue digestion for 15 minutes. Total time (from moment flask is set on heater to completion of digestion) required is about 1 hour.

4. Remove flask from heater and cool to room temperature. When cool, add 100 ml. Reagent 18, Total Nitrogen, from graduated cylinder, washing down neck of Kjeldahl flask. Let stand to cool.

5. Pipette 15 ml. Reagent 16, Total Nitrogen, into a calibrated test tube and immerse test tube in a 500 ml. wide-mouth Erlenmeyer flask containing about  $\frac{3}{4}$  tap water. Set flask and tube on iron support shelf. Arrange this unit and heater unit for the distillation.

6. Add 20 ml. Reagent 17, Total Nitrogen, to the Kjeldahl flask. Attach the

connecting bulb assembly, stoppering "trap-end" tightly into the flask. The outlet tube is put into the test tube with the tip momentarily withheld above the level of the Reagent 16, Total Nitrogen, while the mixture in the Kjeldahl flask is stirred thoroughly. After mixing, the tip of the distillation outlet tube is immersed in the 15 ml. of Reagent 16, Total Nitrogen. The flask is placed on the heater with the current connected.

7. Distillation is continued until the distillate reaches the level marked on the test tube, whence the water flask and tube are immediately removed from the distillation outlet. The heat may then be turned off and the connecting bulb assembly removed.

8. When the distillate has cooled to room temperature the volume is made exactly to 50 ml. with Reagent 18, Total Nitrogen. The solution is mixed thoroughly.

9. Pipette 5 ml. aliquots into each of 2 comparison vials. Add 1 ml. Reagent 6, Nitrogen, into each tube. Let stand 1 minute. Stopper and mix well.

10. Compare with *Soil Ammoniacal Standards*, using the illuminator. Refer to the table for results.

11. When the result is indicated to be above that shown in the first column of data, diluted solutions may be made as indicated on the chart. Five ml. of diluted solution are taken for analysis.

Results obtained from the above test include organic and ammoniacal nitrogen.

TABLE FOR N IN MILL WATER (ORGANIC AND AMMONIACAL N)

Parts per million (p.p.m.) N and pounds per million gallons (lb./mil. gal.)

Tube No.	5 ml. Orig. Distillate		10 ml. Orig. Distillate 10 ml. Reagent 18, T N Take 5 ml. Aliquots		10 ml. Orig. Distillate 30 ml. Reagent 18, T N Take 5 ml. Aliquots	
	p.p.m.	lb./mil. gal.	p.p.m.	lb./mil. gal.	p.p.m.	lb./mil. gal.
1	..	...	..	...	..	...
2	0.5	4.2	1.1	9.1	2.4	19.9
3	0.9	7.5	1.9	15.8	4.0	33.2
4	1.3	10.8	2.7	22.4	5.6	46.5
5	1.7	14.1	3.5	29.0	7.2	59.8
6	2.3	19.1	4.7	39.0	9.6	79.7
7	2.9	24.1	5.9	49.0	12.0	99.6
8	3.9	32.4	7.9	65.6	16.0	132.8

## II. Nitrate Nitrogen:

To determine nitrate nitrogen, use the nitrate nitrogen in irrigation water method.

### RAPID DETERMINATION OF NITROGEN IN IRRIGATION WATERS

(Water Other Than Mill Waters or Waters Containing Organic Matter)

Use the same equipment as is required in making determinations of available nitrogen in soils.

### General Procedure:

Collect 1 gallon of water. Mix thoroughly and filter sufficient amount for analysis (approximately 250 ml.). Use Whatman No. 12, 15-cm. filter paper.

*I. To Determine Ammoniacal Nitrogen in Irrigation Water:*

1. Pipette 5 ml. filtered sample into each of 2 test vials.
  2. Add 1 ml. Reagent 6, Nitrogen. Let stand 1 minute. Stopper and mix well.
  3. Compare with *Soil Ammoniacal Nitrogen Standards*, using the illuminator.
- Refer to table for results.

AMMONIACAL NITROGEN IN IRRIGATION WATER

Std. No.	p.p.m. N	lb N/mil. gal.
1	0.4	3
2	1.2	10
3	2.0	17
4	2.8	23
5	3.6	30
6	4.8	40
7	6.0	50
8	8.0	67

*II. To Determine Nitrate Nitrogen in Irrigation Water:*

1. Pipette 25 ml. of the filtered water into a 100-ml. beaker and evaporate nearly to dryness on hot plate at low heat.
2. When cool, add 2 ml. Reagent 7, Nitrogen, using special pipette.
3. Mix well by means of glass rod, breaking up the residue to insure its intimate contact with the reagent. Let stand 5 minutes.
4. Add from a graduated cylinder 10 ml. Reagent 8, Nitrogen, and stir well to effect solution.
5. Add 15 ml. Reagent 9, Nitrogen, stirring the solution in the beaker while Reagent 9, Nitrogen, is being added. (Add by using 25 ml. graduated cylinder or dispensing burette.) Let final solution cool.
6. Decant liquid into 2 test vials. Compare with *Soil Nitrate Nitrogen Standards*, using the illuminator. The color tubes are designated as 1 to 8, beginning with the lowest standard. Refer to table for results.

NITRATE NITROGEN IN IRRIGATION WATER

Std. No.	p.p.m. N	lb N/mil. gal.
1	0.4	3
2	1.0	8
3	2.0	17
4	3.0	25
5	4.0	33
6	6.0	50
7	8.0	67
8	10.0	83

RAPID ESTIMATION OF NITROGEN IN CANE JUICE

*Equipment Required*

- 4 Supports, iron, 6 in. x 9 in., with rods 15 in.
- 2 Heaters, precision, electric.

- 2 Clamps, burette, castaloy, large, with asbestos-covered jaws.
- 2 Support shelves, round.
- 6 Flasks, Kjeldahl, 300-ml. cap.
- 1 Box for Kjeldahl flasks.
- 1 Rack, test tube.
- 4 Flasks, Erlenmeyer, wide mouth, 500 ml.
- 6 Test tubes, Pyrex, calib. 50 ml., 200 x 29 mm.
- 6 Rubber stoppers, No. 6.
- 2 Connecting bulb assemblies.
- 2 Hengar tubes, micro-size, with filter paper tape.
- 1 Pipette (bacteriological), 1-ml. cap.
- 1 Pipette, special, for Reagent 15, Total N.
- 1 Bottle (g. s. b.), 6 oz.
- 1 Pipette, volumetric, 5-ml. cap.\*
- 1 Pipette, special, to deliver Reagent 6, N, 1-ml. cap.\*
- 1 Burette, special, dispensing, with rubber tube and pinchcock for delivering Reagent 17, Total N.
- 1 Cylinder, graduated, 100-ml. cap.
- 2 Juice screens.
- 1 Clamp, wooden.
- 1 Set ammonia nitrogen in soil color standards.\*
- 1 Vial block.\*
- 1 Phosphate illuminator.\*
- 10 Rubber stoppers, No. 00.\*
- 12 Shell vials, tall form.\*
- 2 oz. Glass beads, perforated.
- 1 Small horn spoon.
- 1 Pipette, volumetric, 15-ml. cap.
- 2 Pyrex beakers, 600-ml. cap.
- 6 Pyrex beakers, 400-ml. cap.
- 6 Pyrex beakers, 250-ml. cap.
- 1 Pipette, volumetric, 25-ml. cap.
- ¼ lb. Potassium sulfate C. P. Powd.
- ½ pt. Reagent 15, Total N.
- 1 qt. Reagent 17, Total N.
- 1 qt. Reagent 16, Total N.
- 1 gal. Reagent 18, Total N.\*
- ¼ pt. Reagent 6, N.\*
- ¼ pt. Cane juice preservative.

\* Note: Standard equipment included in soil phosphate and available soil nitrogen assemblies.

### *Preliminary Procedure*

1. Use fresh, untreated cane juice or juice treated with the proper preservative supplied for rapid chemical methods.
2. Obtain a representative sample. Mix well. Let stand for exactly 15

minutes and then decant about 250 to 500 ml. into a clean 400- or 600-ml. beaker. Pass decanted juice through a juice screen into a 250- or 400-ml. beaker.

3. Immediately before pipetting portions for analysis, mix the screened juice thoroughly.

#### DETERMINATION OF TOTAL NITROGEN

1. (a) Pipette 1 ml. of screened juice into a 300-ml. Kjeldahl flask, taking care to deliver the juice to the bottom of the flask.

(b) Drop 2 glass beads into the flask.

(c) Add  $1/3$  of a small horn spoonful of potassium sulfate ( $K_2SO_4$ ) powder.

(d) Introduce 4 ml. of Reagent 15, Total N, with the special pipette.

2. Let stand for about 5 minutes. Fit a Hengar tube into the neck of the flask and place the Kjeldahl flask on the heater to digest.

3. Digest for  $1/2$  hour. Using the wooden clamp, remove flask to the box. Cool to room temperature.

4. Add 100 ml. of Reagent 18, Total N, from a graduated cylinder, washing down the neck of the Kjeldahl flask. Let stand to cool.

5. Add 15 ml. of Reagent 16, Total N, to a large calibrated test tube. Immerse in cold water in a 500-ml. Erlenmeyer flask. Set flask and tube on iron support shelf and arrange this unit adjacent to heater unit for the distillation.

6. With the solution in the Kjeldahl flask at room temperature, add 20 ml. Reagent 17, Total N, from the special dispensing burette. Turn on heater current. Immediately attach the connecting bulb assembly, stoppering "trap-end" tightly with the flask. The outlet tube is put into the test tube with the tip momentarily withheld above the level of Reagent 16, Total N, while the mixture in the Kjeldahl flask is stirred thoroughly. After mixing, the tip of the distillation outlet tube is immersed in the 15 ml. of Reagent 16, Total N. Place the Kjeldahl flask on the heater.

7. Distillation is continued until the distillate reaches the level marked on the test tube, when the water flask and tube are immediately removed from the distillation outlet. The heater may then be turned off and the connecting bulb assembly removed. The test tube is removed from the Erlenmeyer flask and placed on the tube rack to cool. Cool distillate to room temperature.

8. Make volume exactly up to the 50-ml. mark with Reagent 18, Total N. Stopper test tube with No. 6 rubber stopper and mix contents thoroughly by inverting several times.

9. Pipette 5 ml. into each of 2 comparison vials. Add 1 ml. Reagent 6, N, to each with the special pipette. Stopper and let stand 1 minute.

10. Compare on the illuminator with the ammonia nitrogen-in-soil color standards.

11. Refer to the table under column headed "5-ml. Aliquot" for result.

12. When the nitrogen content is as high as standard tube No. 7, pipette 25 ml. of the distillate into a clean, dry 250-ml. beaker, add 25 ml. Reagent 18, Total N, from a graduated cylinder and mix well. Proceed with steps Nos. 9 and 10. Refer to the table under the column headed "Diluted solution" for result.

## PERCENTAGE TOTAL NITROGEN (N) IN CANE JUICES

5-ml. Aliquot		Diluted Solution	
Ammon. N. Std. Tube No.	Per Cent Total N in Juice	Ammon. N. Std. Tube No.	Per Cent Total N in Juice
1	Nil	1	...
2	.004	2	...
3	.008	3	...
4	.012	4	.026
5	.016	5	.034
6	.022	6	.046
7	.028	7	.058
8	.038	8	.078

## RAPID CHEMICAL METHODS NITROGEN STUDIES

The following is an outline which may be employed as a guide in making nitrogen studies on the plantation.

The details included in the outline embrace :

- I. Plan of experiment.
- II. Collection of samples and preparation for analysis.
- III. Analysis of plant material.

*Note:* Refer to directions for nitrogen in soil and nitrogen in juice for other analytical procedures.

(A modification and simplification of the rapid chemical method for determining nitrogen in plant material is now under study by L. E. Davis.)

### *I. Plan of Experiment:*

*Plan No. 1:* A study by R. C. M. and field experiments of available nitrogen in the soil and of total nitrogen in the cane plant before there is millable cane, followed by total nitrogen in the juice of the millable cane.

1. Within the experimental area, select a sampling station which will include one plot of each treatment in the test. (If the layout is a "block arrangement" this sampling station may be any one "block" of plots.)

2. Within each of the plots in this sampling station, select a definite cane row (not an outside row) and secure a soil sample therefrom by compositing not less than 20 borings taken with a 2-inch auger to a depth of 12 inches about 12 inches away from the center of the cane row, on both sides of the center.

This soil sample should be taken as soon as the seed is planted or the ratoon crop is started, *before* any nitrogen fertilizer is applied. Determine the R. C. M. nitrogen in these soil samples (one for each treatment).

3. Just prior to each nitrogen application that is to be made to the experimental area, select (at random) 2 feet of line within the cane row that was originally sampled in each plot. Take a cane sample by cutting out *all* the cane growing on these 2 feet of line. Up to the time when the cane is about 4 or 5 months old, the entire plant

material itself may be prepared\* and analyzed by R. C. M. for total nitrogen. When there is sufficient millable cane for a juice sample, the cane stalks may be topped, the millable cane crushed, and the crusher juice analyzed for its total nitrogen content. Thereafter, take a soil sample from this 2-foot section of row by compositing the soil from 6 borings, taken as for the original soil sample.

Analyze cane for total N, and soil sample for available N by R. C. M.

4. At harvest, analyze crushed juice of each plot in the sampling station for total nitrogen. Also take a soil sample as in the original sampling and analyze by R. C. M. for available nitrogen.

5. These data will be studied in connection with the results obtained in the experiment.

(Note: Always fill the auger holes that have been bored when taking out the soil sample.)

*Plan No. 2:* A study by R. C. M. of available soil nitrogen and total plant nitrogen at field sampling stations in crop cane.

1. Within any field of crop cane just planted or ratooned, choose a sampling station that will consist of twelve 50- to 60-foot rows of cane, and divide this station for 2 treatments (P. P. and X) into 4 sections as follows:

I 6 rows P. P.	III 6 rows X
II 6 rows X	IV 6 rows P. P.

2. Take 20 soil borings from the 2 center lines of each section and analyze the composited sample by R. C. M. for the amount of available nitrogen that is in the soil at the start of the crop.

3. Apply nitrogen as in the plantation practice to the 2 sections marked "P. P." Omit the nitrogen application on the 2 sections marked "X."

Watch for any definite evidence of slower growth, of any yellowing of leaves, etc., on the "X" plots and when this evidence is secured, take both a cane and a corresponding soil sample for nitrogen analysis from each of the 4 sections.

a. Take the cane sample by cutting out all cane growth from 2 feet of line within one of the 2 center rows; take its corresponding soil sample by making 6 borings with a 2-inch auger to a depth of 12 inches, at a distance of 12 inches from the center of this harvested row on both sides. Analyze both cane and soil for nitrogen content.

b. Definite evidence of slower growth may be secured by marking at least 20 shoots or stalks within the 2 center lines of each section and taking weekly growth measurements thereof.

5. Thereafter several procedures may be followed, depending on conditions:

(a) If no differences are apparent up to the time when the next nitrogen applica-

\* See II. Collection of Samples and Preparation for Analysis.



tion is scheduled, the nitrogen can again be omitted from the "X" plots; etc., until differences do appear.

(b) If differences are apparent early, apply on the "X" plots the nitrogen that was omitted, and see if the cane can pick up its lost growth before the cane on the "P. P." plots is scheduled to get its next nitrogen application.

(c) If differences are not apparent until almost time for the next nitrogen application, make this next application of nitrogen the same to both the "P. P." and the "X" plots, and see if the cane on the "X" plots, which is minus one nitrogen application, can catch up with the cane on the "P. P." plots.

In all cases make determinations of the nitrogen in both the soil and plant whenever differences in cane growth and appearance are apparent.

This procedure should be followed for each scheduled application of nitrogen to the field, *a new sampling station being chosen each time.*

## II. Collection of Samples and Preparation for Analysis:

### (Obtaining a Specimen of Cane for the Determination of Nitrogen)

1. Depending upon the nature of the experiment or the purposes of the study, let it be assumed that a bundle of cane has been cut from a plot or definite area. The entire portion of the plant above ground is to be used for analysis. Obtain green weight, "A," of entire bundle.

2. Flatten out the bundle so that the individual stalks (with leaves attached) lie side by side, one layer deep. Select and reserve every fifth or sixth stalk starting at one end of the flattened bundle. If the sample is very small or the cane is quite young, then *all* of the collected cane may be reserved for analysis. (From  $\frac{1}{2}$  pound to 1 pound of *dry* sample will be sufficient.)

3. The selected cane and leaves should be finely chopped. Mix the chopped material thoroughly and quarter the sample down until but enough remains to fill a 1-pint Mason jar, after it has been ground and dried. Obtain weight, "B," of the reduced portion of finely chopped material. Spread out to dry in a warm, clean room. When dry, grind the sample to about the fineness of granulated sugar, using, if preferred, a grinder furnished by the Chemistry Department which is available with other supplies.

4. Place sample (in tared pan) in electric oven and dry at temperature between  $80^{\circ}\text{C}.$  –  $100^{\circ}\text{C}.$ , preferably  $80^{\circ}\text{C}.$ , until constant weight is obtained.

5. Allow pan containing dried sample to cool and obtain weight, "C," of oven-dry sample. When the sample has been dried to constant weight at the indicated temperatures, it is then assumed that the sample is moisture free. This will eliminate the determination of moisture for the calculation of results to moisture-free basis. Proceed with step No. 4, rapid determination of total N in plant material.

6. To calculate total nitrogen in the cane as it stood in the field:

$$(1) \frac{A}{B} \times C = D \text{ Total moisture-free material in original bundle harvested}$$

- (2)  $D \times \text{per cent Total N} = E$  (Amount in pounds or grams of total nitrogen in the cane as harvested, original bundle.)

Convert the value "E" by appropriate calculation into pounds nitrogen per acre or per line of known length.

### *III. Analysis of Plant Material:*

(Rapid Determination of Total Nitrogen in Plant Material)

1. Obtain representative sample.
2. Chop green material and dry it to moisture-free condition.

(Consult "II. Collection of Samples and Preparation for Analysis" at this point.)

3. Grind dry sample, mix thoroughly and redry to moisture-free state.
4. Weigh accurately 2.5-gm. oven-dry sample and transfer to Kjeldahl flask, R. C. M. Drop in 4 glass beads. Add 1 to 2 grams of special potassium sulfate. Introduce 20 ml. Reagent 15, N, with graduated cylinder. Let stand 5 minutes.
5. Place Kjeldahl flask on heater to digest. Insert gas absorption Hengar tube into neck of flask.
6. Digest for *one* hour or until solution is clear and oxidation process is completed (very pale, straw color).
7. Cool to room temperature. Add 100 ml. Reagent 8, N, from graduated cylinder, washing down neck of Kjeldahl flask. Cool slightly. Transfer by pouring into 250-ml. volumetric flask through a 65-mm. funnel. Rinse Kjeldahl flask several times with Reagent 8, N, transferring the rinsings to the 250-ml. volumetric flask. When the solution in the volumetric flask is cool, make volume up to 250-ml. mark with Reagent 8, N. Stopper and mix thoroughly.
8. Pipette 10 ml. of the solution from the volumetric flask into a clean Kjeldahl flask. Drop in 2 glass beads. Add 100 ml. Reagent 8, N, and 20 ml. Reagent 17, N. Connect flask with distillation apparatus. Shake flask slightly. Immediately place Kjeldahl flask on heater and introduce distillation outlet into the calibrated test tube to which has been added 15 ml. Reagent 16, N. The test tube is immersed in cold water in a 500-ml. Erlenmeyer flask which acts as a condenser.
9. Remove test tube when nearly 50 ml. have been distilled over. Cool distillate to room temperature. (Tube is removed before heat is shut off from the distilling flask.)
10. Make volume to exactly 50 ml. with Reagent 8, N. Mix thoroughly.
11. Pipette 5 ml. into comparison vial. Add 1 ml. Reagent 6, N; let stand 1-minute. Stopper and mix well. (The original distillate may be used or dilutions of the original may be made. However, for both original distillate or diluted aliquots, 5-ml. portions are used for color development. The dilution should be thoroughly mixed before the 5-ml. portions are withdrawn for color development with Reagent 6, N.)
12. Compare with *Soil Ammoniacal Standards*, using the illuminator. Refer to table for results.

## DATA FOR TOTAL N IN PLANT MATERIAL

2.5-gm. Sample — 250 ml. Digested Soln.  
 10-ml. aliquot used in distillation, equivalent to 0.1-gm. Sample.  
 50 ml. Distillate Obtained.

PER CENT TOTAL N IN PLANT MATERIAL, USING GROUND  
OVEN-DRY SAMPLE

\*Distillate Diluted as Indicated Below:

Reading Tube No.	5 ml. Orig. Distillate	5 ml. Orig. Dist. 5 ml. Reag. 8	5 ml. Orig. Dist. 15 ml. Reag. 8	5 ml. Orig. Dist. 35 ml. Reag. 8
1	..	..	..	..
2	.06	.12	..	..
3	.10	.20	.40	.80
4	.14	.28	.56	1.12
5	.18	.36	.72	1.44
6	.24	.48	.96	1.92
7	.30	.60	1.20	2.40
8	.40	.80	1.60	3.20

\* Use 5 ml. of the final solution, diluted in proportions as indicated, for color development.

*Additional Equipment Required*

Six only 250-ml. volumetric flasks, with rubber stoppers, pans for holding and drying sample, chopping knives, balance, oven and grinding machine.

## RAPID ESTIMATION OF TOTAL NITROGEN IN MOLASSES

(Sample to be weighed out on an analytical balance)

*Equipment Required*

Items required for nitrogen-in-juice determinations.

1 Flask, volumetric, 100-ml. cap.

*Procedure*

1. Weigh out 5.0 grams of molasses in a weighing dish or small tared beaker.
2. Add about 25 ml. distilled water (N-free) and mix well by stirring.
3. Transfer to a 100-ml. volumetric flask, washing in with distilled water. Make volume up to the mark, stopper the flask and mix.
4. Proceed according to the directions for the rapid estimation of nitrogen in cane juices, steps Nos. 1-10, taking 1 ml. of the molasses solution *immediately* after mixing.
5. Refer to the table below under column headed "5-ml. Aliquot" for the result.
6. When the nitrogen content is as high as standard tube No. 7, pipette 25 ml. of the distillate into a clean, dry, 250-ml. beaker, add 25 ml. of distilled water (N-free) from a graduated cylinder and mix well. Proceed with steps Nos. 9 and 10, Rapid Estimation of Nitrogen in Cane Juices, and refer to the table below under the column headed "Diluted Solution" for the result.

TABLE FOR TOTAL NITROGEN IN MOLASSES

Ammon. N. Std. Tube No.	5-ml. Aliquot		Diluted Solution	
	Per Cent	lb/Ton	Per Cent	lb/Ton
2	.08	1.6	..	..
3	.16	3.2	..	..
4	.24	4.8	.52	10.4
5	.32	6.4	.68	13.6
6	.44	8.8	.92	18.4
7	.56	11.2	1.16	23.2
8	.76	15.2	1.56	31.2

## RAPID ESTIMATION OF PHOSPHATE IN SOILS

*Equipment Required*

- 1 Electric hot plate.
- 12 Flasks, Erlenmeyer, 125 ml.
- 12 Funnels, 65 mm.
- 1 Funnel support.
- 12 Beakers, Pyrex, 50-ml. cap.
- 24 Vials, shell, tall form.
- 1 pkg. No. 3 Munktell filter paper, 11 cm.
- 1 Pipette, volumetric, Exax, 10-ml. cap.
- 2 Burettes, dispensing, 250-ml. cap.
- 2 Covers for 250-ml. dispensing burettes.
- 2 Dropping bottles, with T. K. covers, 30-ml. cap.
- 1 Dropping bottle, pipette stopper with nipple, 30-ml. cap.
- 1 Metal soil cup, 10-gram cap.
- 1 Spatula, stainless steel, 4-inch blade.
- 1 Vial block.
- 1 Phosphate illuminator.
- 3 Supports, iron, 6 in. x 9 in.
- 1 Clamp, burette, Lincoln.
- 2 Clamps, burette, castaloy, large, with rubber-covered jaws.
- 1 Box containing phosphate color standards (X and Y and High-Register Standards).
- 1 gal. N/2 hydrochloric acid solution.
- 1 gal. Reagent 4,  $P_2O_5$ .
- 1/2 pt. Stannous chloride solution, in g. s. b.
- 1/2 lb. Conc. nitric acid, special, in g. s. b.
- 1/2 lb. Conc. hydrochloric acid, special, in g. s. b.

*Procedure*

1. Fill a 10-gram metal cup with prepared soil and level off with a stainless steel spatula. Transfer to a 125-ml. Erlenmeyer flask.
2. Add 30 ml. of N/2 hydrochloric acid solution from a 250-ml. dispensing burette.

3. Immediately swirl for  $\frac{1}{2}$  minute.
4. Immediately filter through No. 3 Munktell filter paper, 11 cm., into a 50-ml. beaker.
5. Transfer a 10-ml. portion of the filtrate with a pipette to another 50-ml. beaker.
6. Add 10 drops of conc. nitric acid from a dropping bottle with T. K. cover.
7. Evaporate to dryness on an electric hot plate at a low heat. *Do Not Bake.*
8. Add 10 drops of conc. nitric acid and 10 drops of conc. hydrochloric acid from dropping bottles with T. K. covers. Evaporate to dryness.
9. *Important:* If the residue is discolored, repeat the treatment with nitric acid and hydrochloric acid, followed by evaporation. If discoloration remains, repeat once more. If residue is still dark, disregard the discoloration and proceed.
10. Add 10 drops of conc. hydrochloric acid and evaporate to dryness. Repeat the addition of conc. hydrochloric acid and evaporation to dryness. *Do Not Bake.*
11. As soon as the residue is cool add 10 ml. of Reagent 4,  $P_2O_5$ , from a 250-ml. dispensing burette and swirl the contents of the beaker carefully for a few minutes to dissolve the residue. If the residue does not dissolve readily allow the mixture to stand for a while (with occasional swirling), but not longer than 15 minutes. If there is still a residue, disregard it and proceed.
12. Transfer to a phosphate vial, filling to about  $\frac{1}{4}$  inch from the top. If there is a residue, transfer the supernatant liquid but not the residue.
13. Place the phosphate vial in a numbered vial block. Add stannous chloride, one drop at a time. Following each addition mix well, immediately place the vial in the phosphate illuminator and compare with the phosphate color standards, choosing that one of the 3 sets which best matches the hue and intensity of the solution in the vial. Continue until a maximum color intensity has been obtained.
14. Estimate the phosphate content of the soil by interpolation, referring to the attached chart and the table for high-register  $P_2O_5$  standards. Record the data as "Low," "Doubtful," etc., as percentage of  $P_2O_5$  and as pounds of  $P_2O_5$  per acre-foot of soil. Note that each range (Low, Doubtful, etc.) includes the color standard tube so marked and all colors *more* intense up to, but not including, the next color standard. Colors in the high-register range are recorded as "High." For example:

"X" or "Y" {	.0014 per cent = Doubtful
	.0015 per cent = Medium
	.0016 per cent = Medium
High Register .009 per cent = High	

15. Should the color developed be *equal to or greater* than High-Register  $P_2O_5$  Standard VII, transfer  $\frac{1}{2}$  of the solution in the vial to another vial and add an equal volume of Reagent 4,  $P_2O_5$ .
16. Mix well and add 1 drop of stannous chloride solution. Compare with the high-register standards. Refer to the figures under "After Dilution" in the table and record as "High," per cent  $P_2O_5$  and pounds  $P_2O_5$  per acre-foot.
17. When the color is still darker than Standard VII, report the result as "High," greater than .032 per cent, greater than 800 pounds  $P_2O_5$  per acre-foot, or  

>.032 per cent
>800 Lbs./a-ft.

### Precautions

1. Store stannous chloride solution, Reagent 4,  $P_2O_5$ , in a dark place.
2. Color standards should be kept in closed boxes when not in actual use.
3. When the small pieces of tin in the bottles of stannous chloride solution disappear, reject the solution and obtain fresh supplies. Order this reagent in small quantities.

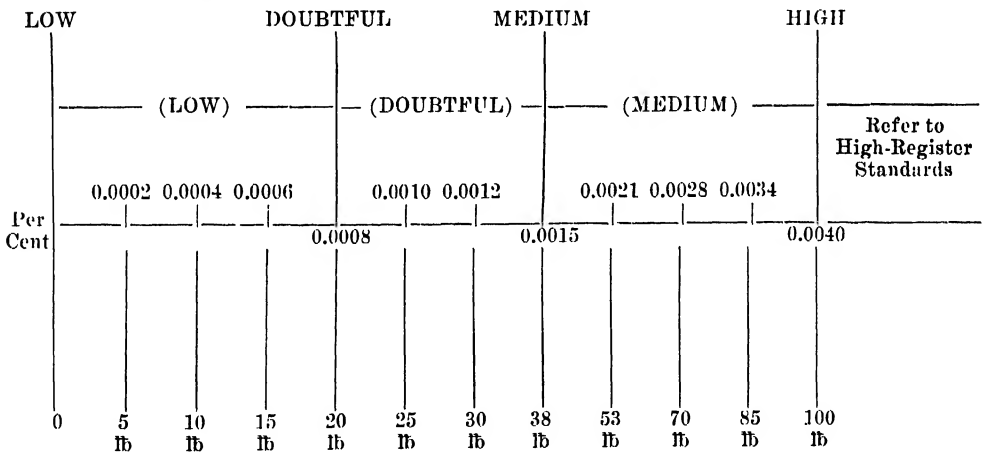
TABLE FOR HIGH-REGISTER SOIL  $P_2O_5$  STANDARDS

Standard No.	I		II		III		IV		V		VI		VII	
$P_2O_5$ Values	Per Cent	lb/ a-ft.	Per Cent	lb/ a-ft.	Per Cent	lb/ a-ft.	Per Cent	lb/ a-ft.	Per Cent	lb/ a-ft.	Per Cent	lb/ a-ft.	Per Cent	lb/ a-ft.
	.004	100	.006	150	.008	200	.010	250	.012	300	.014	350	.016	400

### AFTER DILUTION

One volume of developed solution plus one volume of Reagent 4, $P_2O_5$ .	.016	400	.020	500	.024	600	.028	700	.032	800
Color intensity greater than VII should be recorded as "greater than .032 per cent, 800 pounds, per acre-foot," or (>.032 per cent, >800 pounds per acre-foot).										

INTERPOLATED PERCENTAGE FIGURES FOR  $P_2O_5$  (R. C. M.) AND THEIR EQUIVALENTS IN POUNDS OF  $P_2O_5$  PER ACRE-FOOT



### PHOSPHATE FIXATION

#### Equipment Required

- 1 Support, iron, 6 in. x 9 in.
- 1 Dropping bottle, pipette stopper with nipple, 30-ml. cap.
- 12 Prescription ovals, 2 oz., etched.
- 12 Vials, shell, tall form.
  - 1 Set phosphate fixation color standards, in box (include pc. of black sateen).
  - 1 Metal soil cup, 5-gram cap.
- 2 pkg. Munktell No. 3, 11-cm. filter paper.
- 1 Burette, dispensing, 250-ml. cap.

- 1 Burette cover, for 250-ml. dispensing burette.
- 1 Pipette, Exax, volumetric, 1-ml. cap.
- 1 Vial block.
- 1 Spatula, stainless steel, 4-inch blade.
- 1 Funnel support.
- 1 gal. Series 100 fixation solution.
- 1 gal. Series 500 fixation solution.
- 1 gal. Series 1000 fixation solution.
- 1 pt. Ammonium molybdate solution.
- ½ pt. Stannous chloride solution in g.s.b.
- 1 Clamp, burette, castaloy, large, with rubber-covered jaws.
- 12 Funnels, 65 mm.

#### *Additional Equipment Recommended*

- 2 Burettes, dispensing, 250-ml. cap. (with proper supports).
- 2 Covers, for 250-ml. dispensing burettes.

#### *Equipment Required for Phosphate Fixation Not Included in Potash or Phosphoric Acid Ensembles*

- 12 Prescription ovals, 2 oz., etched.
- 1 Set phosphate fixation color standards, in box (include pc. of black sateen).
- 1 Pipette, Exax, volumetric, 1-ml. cap.
- 1 gal. Series 100 fixation solution.
- 1 gal. Series 500 fixation solution.
- 1 gal. Series 1000 fixation solution.
- 1 pt. Ammonium molybdate solution.

#### *Preliminary*

Three series of phosphate fixation solutions are provided which vary in concentration with respect to phosphoric acid. Since each of these solutions is applied in the same volume to the same quantity of soil it represents varying applications of phosphate fertilizer to the field, as follows:

Series 100	—	1,500 lb $P_2O_5$ per acre-foot
Series 500	—	7,500 lb $P_2O_5$ per acre-foot
Series 1000	—	15,000 lb $P_2O_5$ per acre-foot

*I. Series 100:* When unfamiliar with the general range of phosphate fixing power of the soils to be studied, or whenever general experience has shown that the soils of a region frequently yield index values less than 90 with Series 100 solution, it is desirable to employ this solution. In cases where Series 100 solution almost invariably gives index values of 90, it is not necessary to start with this fixation solution.

*II. Series 500:* Whenever the Series 100 index value is 60 or higher, make additional determinations with Series 500 solution. If the Series 500 index is below 20, use Series 100 solution if that has not already been done.

*III. Series 1000:* Whenever the Series 500 index value is 40 or higher, determinations should be made with the Series 1000 solution.

#### *Procedure*

1. Transfer 30 ml. of the proper phosphate solutions from a 250-ml. dispensing burette to a labeled bottle.
2. Fill a 5-gram metal cup with prepared soil and level off with a stainless steel spatula.
3. Transfer the soil to the bottle containing phosphate solution, and proceed:
  - (a) With Series 100: Shake 1 minute and let stand overnight. Shake 1 minute again before filtering.
  - (b) With Series 500 and 1000: Shake 3 minutes; let stand for 24 hours; shake 3 minutes again; let stand another 24 hours and finally (after a total duration of 48 hours) shake 3 minutes before filtering.
4. Filter through No. 3 Munktell filter paper, 11 cm., into a clean shell vial, tall form. Discard approximately the first 5 ml. and then collect filtrate in the tube until it is about an inch from the top of the tube.
5. Place the vials in a numbered vial block and add 1 ml. ammonium molybdate solution, mix and add 1 drop of stannous chloride solution. Mix well; let stand  $\frac{1}{2}$  minute, or until maximum color is developed.
6. Place on phosphate illuminator and compare with phosphate fixation color standards.
7. Record index value *with series number* of phosphate solution, e.g., if index number is 20 when Series 100 is used, the recorded item becomes: 100-20.

#### *Precautions*

1. Store stannous chloride solution in a dark place.
2. Color standards should be kept in closed boxes when not in actual use.
3. When the small piece of tin in the bottle of stannous chloride solution disappears, reject the solution and obtain fresh supplies. Order this reagent in small quantities.

### RAPID ESTIMATION OF PHOSPHATE IN CANE JUICE

#### *Equipment Required*

- 12 Funnels, short stem, 90 mm.
- 1 Funnel support.
- 12 Beakers, Pyrex, 50-ml. cap.
- 24 Vials, shell, tall form.
- 1 Burette, dispensing, 250-ml. cap.
- 1 Cover for 250-ml. dispensing burette.
- 1 Support, iron, 6 in. x 9 in.
- 1 Clamp, burette, castaloy, large, with rubber-covered jaws.
- 1 Pipette, special, 0.5-ml. cap.
- 1 Box No. 12, Whatman folded filter paper, 18.5 cm.
- 1 Bottle, dropping, pipette stopper with nipple, 30-ml. cap.



- 1 Set phosphate-in-juice color standards (with box).
- 1 Phosphate illuminator.
- 2 Pipettes, volumetric, Exax, 5-ml. cap.
- 1 gal. Reagent 4,  $P_2O_5$ .
- $\frac{1}{2}$  pt. Stannous chloride solution in g. s. b.
- 1 qt. Distilled water.
- $\frac{1}{4}$  lb. Cane juice preservative.

*Equipment Required for Phosphate-in-Cane-Juice Estimations Not Included in Soil Phosphate or Potash Ensembles*

- 12 Funnels, short stem, 90 mm.
- 1 Pipette, transfer, 0.5 ml. cap.
- 1 Box No. 12 Whatman folded filter paper, 18.5 cm.
- 1 Set phosphate in juice color standards (in box).
- 2 Pipettes, volumetric, Exax, 5-ml. cap.
- 1 qt. Distilled water.
- $\frac{1}{4}$  lb Cane juice preservative.

*Procedure*

Cane juice should either be fresh or preserved with proper preservative, supplied for the rapid chemical methods.

1. Filter cane juice through Whatman No. 12, 18.5-cm. folded filter paper.
2. With the pipette, deliver into a phosphate vial an 0.5-ml. aliquot of filtrate.
3. Then, from the dispensing burette, add Reagent 4,  $P_2O_5$ , to a volume of about 5 mm. from the top.
4. Shake well and add 1 drop of stannous chloride solution.
5. Shake again and immediately compare with the phosphate color standards for cane juice, using the  $P_2O_5$  illuminator. Note result. Then add another drop of stannous chloride solution, shake and make comparison again.
6. If the color is too dark for comparison, use a smaller aliquot until a comparable color is obtained.
7. Refer to the table and by means of the aliquot taken and its  $P_2O_5$  equivalent represented by the color standard, obtain the per cent  $P_2O_5$  by volume in the juice, recording the result indicated by the maximum color developed.
8. If the juice contains more than .064 per cent  $P_2O_5$ , mix 5 ml. of the filtered juice with an equal volume of distilled water, using a 5-ml. pipette. Proceed with steps Nos. 2 to 6. Refer to the table and multiply the value by 2 to obtain the percentage of  $P_2O_5$ .
9. If the value obtained by following step No. 8 is in excess of .128 per cent  $P_2O_5$ , mix 5 ml. of the filtered juice with 10 ml. of distilled water and proceed. Multiply the value in the table by 3.

*Precautions*

1. Store stannous chloride solution and Reagent 4,  $P_2O_5$ , in a dark place.
2. Color standards should be kept in closed boxes when not in actual use.

3. When the small pieces of tin in the bottles of stannous chloride solution disappear, reject the solution and obtain fresh supplies. Order this reagent in small quantities.

PERCENTAGE  $P_2O_5$  BY VOLUME IN CANE JUICE  
Ml. of Cane Juice Taken

Stand No.	0.5	0.4	0.3	0.2	0.1
1	0.006	0.008	0.011	0.016	0.032
2	0.010	0.012	0.016	0.024	0.048
3	0.013	0.016	0.021	0.032	0.064
4	0.016	0.020	0.027	0.040	0.080
5	0.019	0.024	0.032	0.048	0.096
6	0.022	0.028	0.037	0.056	0.112
7	0.026	0.032	0.043	0.064	0.128
8	0.029	0.036	0.048	0.072	0.144

#### RAPID ESTIMATION OF PHOSPHORIC ACID IN BOILER WATER

1. Filter the collected specimen twice through Whatman No. 12, folded filter paper.
2. Transfer 2 ml. of the cold filtrate into a tall form, phosphate vial.
3. Make up the volume to about  $\frac{1}{4}$  inch from the top by adding reagent No. 40.
4. Mix the contents of vial thoroughly by inverting several times, stoppering the vial with the index finger.
5. Add 1 drop of stannous chloride solution.
6. Mix well and immediately compare with the phosphate color standards for cane juice, using the phosphate illuminator. Note result.
7. Add another drop of stannous chloride solution, shake and make comparison again.
8. Consult the table and by referring to the number of the standard which matches the maximum color developed, obtain the concentration of phosphoric acid in the sample tested.

TABLE FOR PHOSPHORIC ACID IN BOILER WATER

Standard No.	P.P.M. $P_2O_5$
1	16
2	24
3	32
4	40
5	48
6	56
7	64
8	72

#### RAPID ESTIMATION OF PHOSPHORIC ACID IN FILTER CAKE

*Note:* The equipment listed below is already part of R.C.M. assemblies for determining  $P_2O_5$  in soil and in crusher juice and nitrogen in soil.

*Equipment Required*

- 1 Only 5-gram metal soil cup.
- 6 Only 125-ml. Erlenmeyer flasks.
- 6 Only 65-mm. glass funnels.
- 1 Only filtering rack.
- 1 Only 250-ml. dispensing burette for N/2 HCl solution.
- 1 Only 250-ml. dispensing burette for Reagent 4,  $P_2O_5$ .
- 1 Only iron support with 2 large clamps for 250-ml. dispensing burettes.
- 6 Only 100-ml. Pyrex beakers.
- 1 Only 0.5-ml. special juice pipette.
- 1 doz. Phosphate vials.
- 1 box Whatman No. 12, 15-cm. filter paper.
- 1 gal. N/2 hydrochloric acid solution.
- 1 qt. Reagent 4,  $P_2O_5$ .
- $\frac{1}{4}$  pt. Stannous chloride solution.
- 1 Only 30-ml. dropping bottle, pipette stopper with nipple, for stannous chloride solution.
- 1 Set phosphate-in-cane-juice color standards.
- 1 Phosphate illuminator.
- 1 Block for holding phosphate vials.
- 1 Only 10-ml. Exax volumetric pipette.

*Procedure*

1. Obtain a representative sample of the filter cake.
2. Break up large lumps by crumbling with hand. Mix material thoroughly. The sample is now ready for analysis without drying.
3. Fill a 5-gram metal soil cup with filter cake. Pack cup by pressing slightly the material with blade of spatula. Scrape off excess specimen to level of cup. Transfer specimen into a 125-ml. Erlenmeyer flask.
4. Add 50 ml. N/2 hydrochloric acid solution. Shake 1 minute and let stand 1 hour.
5. At the end of 1 hour, shake for 1 minute and filter through Whatman No. 12, 15-cm. filter paper, collecting the filtrate in a 100-ml. Pyrex beaker. (Same type of glassware and filter paper as used for available N in soil determination.) Mix the filtrate by stirring.
6. With a special 0.5-ml. juice pipette, deliver in each of 2 phosphate vials, 0.2-ml. aliquots of filtrate.
7. From a dispensing burette, add Reagent 4,  $P_2O_5$ , to a volume of about  $\frac{1}{2}$  inch from the top of the vial. Mix well by inverting tubes several times.
8. Develop color by adding 1 drop of stannous chloride reagent to each vial. Mix well and immediately compare with  $P_2O_5$ -in-cane-juice color standards, using the illuminator.
9. If the color is too dark or too light for comparison, use a smaller or larger aliquot until the shade developed is within range of the standards.
10. Refer to Table A and with reference to the aliquot taken, obtain the pounds  $P_2O_5$  per cubic yard of filter cake.

11. When the phosphate content is above that indicated in Table A, further analysis may be made by diluting a portion of the original filtrate.

12. To proceed with further analysis: Pipette 10 ml. of the filtrate into a 125-ml. Erlenmeyer flask. Add 40 ml. N/2 hydrochloric acid solution, mix solution thoroughly. Use diluted solution for analysis as in steps Nos. 6 to 9, inclusive. Refer to Table B and with reference to the aliquot of diluted solution taken, obtain the pounds  $P_2O_5$  per cubic yard of filter cake.

#### POUNDS $P_2O_5$ PER CUBIC YARD OF FILTER CAKE

TABLE A

(Using Original Filtrate)

Std. No.	ml. of Original Filtrate Used For Analysis				
	0.5	0.4	0.3	0.2	0.1
1	1.0	1.3	1.7	2.7	5.4
2	1.7	2.0	2.7	4.0	8.1
3	2.0	2.7	3.4	5.4	10.8
4	2.7	3.4	4.4	6.7	13.5
5	3.0	4.0	5.4	8.1	16.2
6	3.7	4.7	6.1	9.4	18.9
7	4.4	5.4	7.1	10.8	21.6
8	4.7	6.1	8.1	12.1	24.3

TABLE B

(Using Diluted Filtrate)

Std. No.	ml. of Diluted Filtrate Used For Analysis				
	0.5	0.4	0.3	0.2	0.1
1	5.4	6.7	9.0	13.5	27.0
2	8.1	10.1	13.5	20.2	40.4
3	10.8	13.5	18.0	27.0	53.9
4	13.5	16.8	22.5	33.7	67.4
5	16.2	20.2	27.0	40.4	80.9
6	18.9	23.6	31.5	47.2	94.4
7	21.6	27.0	35.9	53.9	107.8
8	24.3	30.3	40.4	60.7	121.3

#### RAPID ESTIMATION OF POTASH IN SOILS

##### *Equipment Required*

- 1 Metal soil cup, 2.5-gram cap.
- 1 Metal soil cup, 5.0-gram cap.
- 1 Metal soil cup, 10-gram cap.
- 1 Burette, dispensing, 250-ml. cap.
- 1 Cover for 250-ml. dispensing burette.
- 24 Flasks, Erlenmeyer, 125-ml. cap.
- 24 Beakers, Pyrex, 50-ml. cap.
- 24 Funnels, short stem, 65 mm.
- 2 pkg. Munktell No. 3, 9-cm. filter paper.

- 2 pkg. Munktell No. 3, 11-cm. filter paper.
- 24 Vials, shell, short form.
  - 1 Pipette, medicine dropper, 1-ml. cap. (calib.).
  - 1 Burette, Exax, 50-ml. cap.
  - 1 Cover for 50-ml. burette.
  - 4 Inclined blocks.
  - 1 Potash rotator, electric, Model "G."
  - 1 Potash Illuminator.
  - 2 Supports, iron, 6 in. x 9 in.
  - 1 Clamp, burette, Lincoln.
  - 1 Clamp, burette, castaloy, large, with rubber-covered jaws.
  - 1 Funnel support.
  - 1 Spatula, stainless steel, 4-inch blade.
  - 1 Dropping bottle, amber, pipette stopper with nob at tip, 30-ml. cap.
  - 1 gal. Reagent 1,  $K_2O$ .
  - $\frac{1}{4}$  pt. Reagent 2,  $K_2O$  g. s. b.
  - 1 pt. Reagent 3,  $K_2O$ .

### *Procedure*

1. Fill a 2.5-gram metal cup with prepared soil and level off with a stainless steel spatula.
2. Transfer the soil to a 125-ml. Erlenmeyer flask.
3. Add 10 ml. of Reagent 1,  $K_2O$ , from a 250-ml. dispensing burette.
4. Swirl for  $\frac{1}{2}$  minute.
5. Filter through Munktell No. 3 filter paper, 9 cm., into a 50-ml. beaker.
6. With the calibrated medicine dropper, transfer 1-ml. portions of the filtrate to the bottom of each of 2 vials, short form.
7. Add 4 drops of Reagent 2,  $K_2O$ , to each of the vials and mix thoroughly.
8. Immediately place the vials on an inclined block. Hold the block horizontally, thus keeping the vials inclined at the proper angle. Touch the inner side of each vial with the tip of a 50-ml. burette and allow 1 ml. of Reagent 3,  $K_2O$ , to flow down the side so that very little mixing takes place. The addition of Reagent 3,  $K_2O$ , should require approximately  $4\frac{1}{2}$  to 5 seconds with the rate of flow as uniform as possible. Two distinct liquid layers should be visible. In case the layers are broken or a cloudiness appears between the 2 layers, discontinue and repeat steps Nos. 6 to 8 until 2 distinct liquid layers are obtained.
9. Place the vials in slots Nos. 1 and 3 of the potash rotator.
10. If a second soil extract has been prepared, it may be tested at the same time, using slots Nos. 2 and 4.
11. Start the instrument and stop it exactly 30 seconds later.
12. Allow the vials to stand for about 30 seconds and then transfer to the illuminator.
13. Move the slide back and forth over the lined chart and at the same time sight down through the columns of test liquid.

If heaviest lines cannot be seen, reading is 4.

If heaviest lines can be seen, but medium lines are invisible, reading is 3.

If medium lines can be seen but light lines are invisible, reading is 2.

If light green lines can be seen, reading is 1.

14. If the 2 readings do not agree, repeat the duplicate determinations until 2 or more identical readings are obtained.

15. If 2 or more readings of 2 or 3 are obtained, estimate the  $K_2O$  content by reference to the attached table and average the values obtained.

16. If a reading of 1 is obtained, prepare fresh extracts using successively larger amounts of soil with sufficient quantities of Reagent 1,  $K_2O$ , to obtain 5 ml. or more of filtrate. The desirable amounts to use are indicated in the table. Proceed with steps Nos. 6 to 14 until readings of 2 or 3 are obtained. If a reading of 1 is still obtained using a ratio of 1:1 (20 gm. soil to 20 ml. Reagent 1,  $K_2O$ ), record the potash content as "Low,"  $<.003$  per cent and  $<75$  pounds  $K_2O$  per acre-foot.

17. If a reading of 4 is obtained, prepare fresh extracts of 2.5 gm. of soil to 15, 20, 25, 30, 35 and 40 ml. of Reagent 1,  $K_2O$ , until readings of 2 or 3 are obtained. If a reading of 4 is still obtained with 2.5 grams of soil to 40 ml. of Reagent 1,  $K_2O$ , the potash content can be recorded as "High,"  $>.056$  per cent,  $>1400$  pounds per acre-foot.

#### *Precaution*

Store Reagent 2,  $K_2O$ , in a dark place. Order in small quantities.

**APPROXIMATE PERCENTAGES OF  $K_2O$  AND THEIR CORRESPONDING VALUES  
IN POUNDS OF  $K_2O$  PER ACRE-FOOT**

<u>Ratio</u> Soil : Reagent	Read- ings	Per Cent $K_2O$	Lbs. $K_2O$ per acre-foot	Approx. Group
20 gm. 20 ml.	1	<.003	< 75	—LOW
	2	.003	75	
	3	.004	100	
	4	>.004	>100	
15 gm. 20 ml.	1	<.004	<100	
	2	.004	100	
	3	.005	125	
	4	>.005	>125	
10 gm. 20 ml.	1	<.006	<150	
	2	.006	150	
	3	.007	175	
	4	>.007	>175	
7.5 gm. 20 ml.	1	<.009	<225	—DOUBTFUL
	2	.009	225	
	3	.010	250	
	4	>.010	>250	
2.5 gm. 10 ml.	1	<.012	<300	
	2	.012	300	
	3	.014	350	
	4	>.014	>350	
2.5 gm. 15 ml.	1	<.018	<450	—MEDIUM
	2	.018	450	
	3	.021	525	
	4	>.021	>525	
2.5 gm. 20 ml.	1	<.025	<625	
	2	.025	625	
	3	.028	700	
	4	>.028	>700	
2.5 gm. 25 ml.	1	<.031	<775	—HIGH
	2	.031	775	
	3	.035	875	
	4	>.035	>875	
2.5 gm. 30 ml.	1	<.037	<925	
	2	.037	925	
	3	.042	1050	
	4	>.042	>1050	
2.5 gm. 35 ml.	1	<.044	<1100	
	2	.044	1100	
	3	.049	1225	
	4	>.049	>1225	
2.5 gm. 40 ml.	1	<.050	<1250	
	2	.050	1250	
	3	.056	1400	
	4	>.056	>1400	

## RAPID ESTIMATION OF POTASH IN CANE JUICE

*Equipment Required*

- 12 Funnels, short stem, 90 mm.
- 1 Funnel support.
- 1 box Whatman No. 12, 18.5-cm. folded filter paper.
- 1 Burette, dispensing, 250-ml.
- 1 Cover for 250-ml. dispensing burette.
- 12 Beakers, Pyrex, 50-ml. cap.
- 1 Pipette, transfer, straight type, 1-ml. cap.
- 2 Pipettes, medicine dropper, calibrated to 1 ml.
- 24 Vials, shell, short form.
- 2 Burette, Exax, 50 ml.
- 2 Covers for 50-ml. Exax burette.
- 1 Potash rotator, electric, Model "G."
- 1 Potash illuminator.
- 1 Clamp, burette, castaloy, large, with rubber-covered jaws.
- 1 Bottle, dropping, pipette stopper with nipple, for Reagent 2,  $K_2O$ .
- 2 Supports, iron, 6 in. x 9 in.
- 1 Clamp, burette, Lincoln.
- 1 gal. Reagent 10,  $K_2O$ .
- $\frac{1}{4}$  pt. Reagent 2,  $K_2O$  g.s.b.
- 1 pt. Reagent 3,  $K_2O$ .
- $\frac{1}{4}$  lb Cane juice preservative.

*Additional Equipment Required for Potash in Cane Juice Assembly  
Not Included in Potash and Phosphate in Soil Ensembles*

- 12 Funnels, short stem, 90 mm.
- 1 box Whatman No. 12, 18.5-cm. folded filter paper.
- 1 Pipette, transfer, straight type, 1-ml. cap.
- 1 gal. Reagent 10,  $K_2O$ .

*Procedure*

1. Use fresh, untreated juice or juice preserved with the proper preservative supplied for rapid chemical methods.
2. Obtain a representative sample. Shake well.
3. Pour into a dry filter paper (Whatman No. 12, 18.5 cm. folded) on a 90-mm. funnel and collect in a clean, dry 50-ml. beaker.
4. Transfer 1 drop of juice to the bottom of a clean potash vial and 2 drops to a second vial, using a medicine dropper held vertically.
5. With a calibrated medicine dropper add 1 ml. Reagent 10,  $K_2O$ .
6. Add 4 drops of Reagent 2,  $K_2O$ , to each of the vials and mix, using a few quick shakes.
7. Immediately place the vials on an inclined block. Hold the block horizontally, thus keeping the vials inclined at the proper angle. Touch the inner side of each vial with the tip of a 50-ml. burette and allow 1 ml. of Reagent 3,  $K_2O$ , to flow down the side so that very little mixing takes place. The addition of Reagent 3,



$K_2O$ , should require approximately 5 to 6 seconds with the rate of flow as uniform as possible. Two distinct liquid layers should be visible. In case the layers are broken or a cloudiness appears between the 2 layers, discontinue and repeat steps Nos. 4 to 7 until 2 distinct liquid layers are obtained.

8. Place the vials in slots Nos. 1 and 3 of the potash rotator.

9. If a second sample of juice has been filtered, it may be tested at the same time, using slots Nos. 2 and 4.

10. Start the instrument and stop it exactly 30 seconds later.

11. Allow the vials to stand for about 30 seconds and then transfer to the illuminator.

12. Move the slide back and forth over the lined chart and at the same time sight down through the columns of test liquid.

If heaviest lines cannot be seen, reading is 4.

If heaviest lines can be seen, but medium lines are invisible, reading is 3.

If medium lines can be seen, but light lines are invisible, reading is 2.

If light green lines can be seen, reading is 1.

13. Record the readings. These are preliminary tests to indicate the correct dilutions to use in the following section of the procedure.

14. Transfer 1-ml. portions of the juice into clean 50-ml. beakers and add the volume of Reagent 10,  $K_2O$ , indicated by the table below :

TABLE OF DILUTIONS

1-Drop Vial		2-Drop Vial	
Reading	ml. Reagent 10, $K_2O$	Reading	ml. Reagent 10, $K_2O$
2	20	1	10, or less
3	25	2	15
4	30, or greater	3	15

15. Mix well and transfer 1-ml. portions of the diluted juice into each of 2 potash vials, using a calibrated medicine dropper.

16. Repeat steps Nos. 6 to 12 and record the readings.

17. If the 2 readings do not agree, repeat the duplicate determinations until 2 or more identical readings are obtained.

18. If 2 or more readings of 2 or 3 are obtained, estimate the potash content by reference to the table "Potash in Cane Juices" and average the values obtained.

19. If readings of 1 are obtained, add a smaller volume of Reagent 10,  $K_2O$ , with a second 1-ml. portion of juice, as suggested in the table "Potash in Cane Juices."

20. If readings of 4 are obtained, add a larger volume of Reagent 10,  $K_2O$ , with 1 ml. of the juice.

21. Repeat until 2 or more readings of 2 or 3 are obtained. Refer to the table and average the percentage values indicated.

22. If readings of 4 are obtained with 1 dilution and readings of 1 with the next higher dilution, average the results indicated by the corresponding 2 and 3 readings. For example :

We obtain readings of 1 with 20 ml. of reagent and readings of 4 with 15 ml. of reagent. Average the values .16 per cent and .14 per cent, yielding the result, .15 per cent.

23. If, with no dilution, readings of 1 are obtained, record the result as  $<.01$  per cent  $K_2O$ . If, with an addition of 50 ml. reagent 10,  $K_2O$ , readings of 4 are obtained, record the result as  $>.43$  per cent  $K_2O$ .

#### *Precaution*

Store Reagent 2,  $K_2O$ , in a dark place. Order in small quantities.

TABLE FOR POTASH IN CANE JUICES

ml. Reagent 10, $K_2O$	Per Cent Potash ( $K_2O$ )			
	Reading 1	Reading 2	Reading 3	Reading 4
0	$<.01$	.01	.01	$>.01$
3	$<.03$	.03	.03	$>.03$
5	$<.05$	.05	.05	$>.05$
7	$<.06$	.06	.07	$>.07$
10	$<.08$	.08	.09	$>.09$
12	$<.10$	.10	.11	$>.11$
15	$<.12$	.12	.14	$>.14$
20	$<.16$	.16	.18	$>.18$
25	$<.20$	.20	.22	$>.22$
30	$<.23$	.23	.26	$>.26$
35	$<.27$	.27	.31	$>.31$
40	$<.31$	.31	.35	$>.35$
50	$<.38$	.38	.43	$>.43$

#### RAPID ESTIMATION OF POTASH IN MOLASSES

(Sample to be weighed out on an analytical balance.)

#### *Equipment Required*

Items required for potash-in-juice determinations:

1 flask, volumetric, 100-ml. cap.

#### *Procedure*

1. Weigh out 5.0 grams of molasses in a weighing dish or small tared beaker.
2. Add about 25 ml. distilled water and mix well by stirring.
3. Transfer to a 100-ml. volumetric flask, washing in with distilled water. Make volume up to the mark, stopper the flask and mix.
4. Proceed according to the directions for the rapid estimation of potash in cane juices, steps Nos. 3 to 17.

*Note:* Potash may be determined on molasses using the solution prepared for the rapid estimation of total nitrogen in molasses, step No. 3, *after samples for the nitrogen determinations have been secured.* But do not determine total nitrogen on a filtered sample.

5. Proceed with steps Nos. 18 to 22, referring to the table below and substituting for the example in step No. 22, the following:

We obtain readings of 1 with 20 ml. of reagent and readings of 4 with 15 ml. of reagent. Average the values 3.2 per cent and 2.8 per cent, yielding the result,

3.0 per cent, or the values 64 and 56, yielding the result 60 pounds  $K_2O$  per ton of molasses.

TABLE FOR POTASH IN MOLASSES

ml. Reagent 10, $K_2O$	Reading of 2		Reading of 3	
	Per cent	lb/ton	Per cent	lb/ton
10	1.6	32	1.8	36
12	2.0	40	2.2	44
15	2.4	48	2.8	56
20	3.2	64	3.6	72
25	4.0	80	4.4	88
30	4.6	92	5.2	104
35	5.4	108	6.2	124
40	6.2	124	7.0	140
50	7.6	152	8.6	172

## RAPID ESTIMATION OF POTASH IN IRRIGATION WATER

*Equipment Required*

- 1 Only 6-inch electric hot plate.
- 1 Only 250-ml. graduated cylinder.
- 6 Only 400-ml. Pyrex beakers.
- 6 Only 600-ml. Pyrex beakers.
- 3 Only rubber policemen.
- 6 Only 40-mm. glass funnels.
- 1 Only filtering rack (10 funnels).
- 1 Only 1-ml. pipette, graduated to 1/10 ml.
- 1 pkg. Munktell No. 3, 7-cm. filter paper.
- 1 pkg. Munktell No. 3, 9-cm. filter paper.
- 1 Only 2-ml. Mohr pipette.
- 1 doz. Potash vials.
- 1 Only potash rotator.
- 1 Only potash illuminator.
- 1 gal. Reagent 1,  $K_2O$  solvent.
- 1/4 pt. Reagent 2,  $K_2O$  g.s.b.
- 1 pt. Reagent 3,  $K_2O$ .

*Additional Equipment Required for Potash in Irrigation Water Assembly  
Not Included in Other Rapid Method Ensembles*

- 1 Only 250-ml. graduated cylinder.
- 6 Only 400-ml. Pyrex beakers.
- 6 Only 600-ml. Pyrex beakers.
- 3 Only rubber policemen.
- 1 Only 1-ml. pipette, graduated to 1/10 ml.
- 1 pkg. Munktell No. 3, 7-cm. filter paper.

*Note to Analysts:*

You will observe that this rapid method for determination of potash in irriga-

tion waters is essentially a modification of the standard procedure for estimating potash in soil in which the potash rotator, potash illuminator, and other standard equipment are employed.

Step No. 4 reads: "Add 5, 10 or 15 ml. Reagent 1,  $K_2O$ ." To explain: If the volume of residue remaining is bulky after step No. 2 in the process, or if previous knowledge of the water indicates that the potash content may be high, then the amount of Reagent 1,  $K_2O$ , may be taken as 15 ml. instead of 5 or 10 ml. If, on the other hand, the bulk of precipitate is small or potash content is believed to be reasonably low, then use 5 ml. or 10 ml. of the reagent and proceed as directed. For instance: Were you to be working with a pump water from central Maui, where dry residue is high and potash is also high, you would probably decide to use 15 ml. of Reagent 1,  $K_2O$ . If, on the other hand, you were dealing with Waiahole Ditch water from the island of Oahu, where potash is rather low in concentration and where other salts are equally low (hence small residue on drying), you would find it expedient to employ 5 ml. of Reagent 1,  $K_2O$ , in bringing the potash from the dry residue into solution.

Step No. 5 reads: "Rub sides and bottom of the beaker well, using rubber policeman." The purpose of this step is to insure intimate contact of the residue in the beaker with Reagent 1,  $K_2O$ , the latter being an excellent solvent for potash. In many cases you will find that a portion of the residue will not go into solution. This is to be expected and we believe will not occasion any serious difficulty regarding the accuracy of the test, for Reagent 1,  $K_2O$ , will very effectively dissolve the potash in this test, providing you bring it in intimate contact with the reagent for a few seconds.

In analyzing a mountain water or other irrigation supply which may be very low in potash, a larger volume will have to be taken for evaporation. In some waters we have found it necessary to use as much as 750 ml. in order to obtain enough for making an accurate analysis.

A word of explanation regarding the data sheet accompanying the method of analysis: You will find the data sheet divided into 3 sections, one of which is to be used when 5 ml. of Reagent 1,  $K_2O$ , are employed in Step No. 4, another when 10 ml. of Reagent 1,  $K_2O$ , have been used and the third where 15 ml. of this reagent were required to effect solution of the potash. In each case you will find notations at the top of the data sheet, heading columns of data, in which any one of several amounts of filtrate may have been used, varying from 1.0 ml. in decreasing increments of 0.1 ml. down to and including 0.1 ml. Under these headings you will find data already calculated for readings of "2" and "3" corresponding to potash present in parts per million and pounds per million gallons. A reading in this case (as in the case of the soil and juice potash analyses) represents the designations you give the tests when making your turbidity determination with the potash illuminator. You will note we have omitted readings "1" and "4" from the data sheet. This has been done because of the fact that were you to obtain either one of these values it would necessitate a repetition of the analysis simply because the values lie at the extremities of the lined chart and as a consequence cannot be counted upon always as being absolutely correct. A reading of "1," for instance, may be exactly that value, but in all probability it is much less than "1." Likewise, a reading of "4" may be just "4" in relation to the remainder of the chart, but most probably it is higher than "4." Conversely, a reading of "2" or "3" places the determination in an intermediate

position between the extremes of "1" and "4" and hence may be accepted as reliable. To illustrate the discussion in this paragraph, let us take an hypothetical case: A sample of water is evaporated to dryness and the residue brought into solution with 10 ml. Reagent 1,  $K_2O$ ; 0.6 ml. of the filtrate was used in the test and a reading of "2" was made on the illuminator. You would report, then, that the water contained 5.3 parts per million or 44 pounds of potash as  $K_2O$  per million gallons.

When an amount greater than 250 ml. of irrigation water is used for evaporation preparatory to making the analysis, a factor will have to be employed for reducing the readings on the data sheet. For instance, if 500 ml. water are taken, divide the result obtained by 2; if 750 ml. are used, by 3, etc.

#### RAPID METHOD FOR THE DETERMINATION OF POTASH IN PUMP WATERS

1. Measure out 250 ml. of the water into a 400-ml. beaker.\*
2. Place on hot plate and carefully evaporate to dryness.
3. Cool.
4. Add 5, 10 or 15 ml. Reagent 1,  $K_2O$ .
5. Rub sides and bottom of the beaker well, using rubber policeman.
6. Filter through Munktell No. 3 paper into a 50-ml. beaker. (For 5 ml. Reagent 1,  $K_2O$ , use 7-cm. filter paper. For 10 and 15 ml. Reagent 1,  $K_2O$ , use 9-cm. filter paper.)
7. Transfer 0.5 ml. of the filtrate into a potash vial.
8. Make up to 1 ml. with Reagent 1,  $K_2O$ . Shake.
9. Add 4 drops Reagent 2,  $K_2O$ . Shake 3 times.
10. Add 1 ml. Reagent 3,  $K_2O$ , down the inclined side of the vial.
11. Put vial into the potash rotator and run for  $\frac{1}{2}$  minute.
12. The readings are made identically as for other potash kits, using the potash illuminator.
13. Under the columns for the number of ml. of Reagent 1,  $K_2O$ , added to the residue and the fractional part of a ml. of the filtrate taken, read directly the  $K_2O$  content in parts per million (p.p.m.), or pounds per million gallons.
14. If the reading is 4, take 0.4, 0.3, 0.2 or 0.1 ml. of the filtrate and make up to 1 ml. in each case, using Reagent 1,  $K_2O$ , until 3 and 2 readings are obtained.
15. For "1" reading, use 0.6, 0.7, 0.8, 0.9 or 1.0 ml. portions as above until 2 and 3 readings are obtained.

\* For mountain water, use about 500 ml. or more and proceed as usual.

**RAPID DETERMINATION OF POTASH IN PUMP WATER**  
(250 ml. water evaporated to dryness.)

5 ml. Reagent 1,  $K_2O$ , added to residue and filtered

Reading	$K_2O$ content	ml. of filtrate taken									
		1.0 ml.	0.9 ml.	0.8 ml.	0.7 ml.	0.6 ml.	0.5 ml.	0.4 ml.	0.3 ml.	0.2 ml.	0.1 ml.
2	p.p.m.	1.6	1.8	2.0	2.3	2.7	3.2	4.0	5.3	8.0	16.0
	lb/mil. gal.	13	15	17	19	23	27	33	44	67	133
	p.p.m.	1.8	2.0	2.3	2.6	3.0	3.6	4.5	6.0	9.0	18.0
3	lb/mil. gal.	15	17	19	22	25	30	38	50	75	150
10 ml. Reagent 1, $K_2O$ , added to residue and filtered											
2	p.p.m.	3.2	3.6	4.0	4.6	5.3	6.4	8.0	10.7	16.0	32.0
	lb/mil. gal.	27	30	33	38	44	53	66	89	133	267
	p.p.m.	3.6	4.0	4.5	5.1	6.0	7.2	9.0	12.0	18.0	36.0
3	lb/mil. gal.	30	33	38	43	50	60	75	100	150	300
15 ml. Reagent 1, $K_2O$ , added to residue and filtered											
2	p.p.m.	4.8	5.3	6.0	6.9	8.0	9.6	12.0	16.0	24.0	48.0
	lb/mil. gal.	40	44	50	58	67	80	100	133	200	400
	p.p.m.	5.4	6.0	6.8	7.7	9.0	11.0	13.5	18.0	27.0	54.0
3	lb/mil. gal.	45	50	57	64	75	91	112	150	225	450

NOTE: In all cases reading "1" is less than the respective "2" reading, and reading "4" is greater than the respective "3" reading.

## RAPID ESTIMATION OF POTASH AND PHOSPHORIC ACID IN MILL ASH

*Equipment Required*

- 6 Only 100-ml. Pyrex beakers.
- 1 Analytical balance.\*
- 6 Only 125-ml. Erlenmeyer flasks.
- 6 Only 65-mm. glass funnels.
- 1 Only 250-ml. dispensing burette.
- 1 Only 10-ml. pipette.
- 1 pkg. Munktell No. 3, 12.5-cm. filter paper.\*
- 3 Only medicine droppers calibrated to 1 ml.
- 10 Only short-type, shell vials, 2-dram cap.
- 6 Only 50-ml. Pyrex beakers.
- 1 Only 50-ml. burette.
- 1 Only 1-ml. pipette graduated to 0.01 ml.
- 1 Only 2-ml. Mohr pipette.
- 1 Only potash rotator.
- 1 Only potash illuminator.
- 10 Only tall-type, shell vials marked to hold 8 ml. solution.\*
- 6 Only 50-ml. volumetric flasks.\*
- 6 Only rubber stoppers for volumetric flasks.\*
- 1 Only 0.5-ml. pipette, graduated into 0.5-, 0.4-, 0.3-, 0.2- and 0.1 ml. divisions.
- 1 Only filtering rack.
- 1 Only phosphate vial rack.
- 1 Only phosphate illuminator.
- 1 set phosphate-in-cane-juice color standards.
- 1 Data sheet.\*
- 1 gal. Reagent 1,  $K_2O$  solvent.
- ¼ pt. Reagent 2,  $K_2O$  g. s. b.
- 1 pt. Reagent 3,  $K_2O$ .
- 1 gal. Reagent 4,  $P_2O_5$ .
- 1 gal. N/2 hydrochloric acid.
- 1 gal. distilled water.
- 1 pt. Stannous chloride, g. s. b.

\* Note: Not included in other rapid chemical methods assemblies.

*Preparation of Sample for Analysis*

- A. Obtain representative ash sample (about 250 grams—½ lb.)
- B. Break up sample into powder form as much as possible.
- C. Spread sample out evenly on a clean piece of paper.
- D. Divide sample into 4 equal parts.
- E. Take opposite quarters and mix well.
- F. Repeat steps D and E twice. (The portion to be used for analysis will be equivalent to 1/16 of the original sample.)
- G. Analyze resulting specimen for  $K_2O$  and  $P_2O_5$  by Methods I and II, respectively.

### *I. Determination of Potash in Mill Ash*

1. Weigh 1 gram of sample from (G) and place in a 125-ml. flask.
  2. Add 50 ml. of Reagent 1,  $K_2O$  solvent.
  3. Shake 1 minute.
  4. Filter into a clean, dry beaker through Munktell No. 3, 12.5-cm. filter paper.
  5. Transfer 0.5 ml. of the filtrate to the bottom of a short type, comparison vial.
  6. Make up to a full ml., using Reagent 1,  $K_2O$  solvent.
  7. Add 4 drops of Reagent 2,  $K_2O$ , to one of the vials and mix thoroughly.
  8. Immediately incline vial with contents and let 1 ml. of Reagent 3,  $K_2O$ , flow down the side so that very little mixing takes place. (Two distinct liquid layers indicate correct technic.)
  9. Place the vial in a slot of the potash agitator.
  10. Start the instrument. (If a spring-wound rotator is used, it is important to rewind the spring for every 2 to 4 determinations.)
  11. Run instrument for 30 seconds, the timing to start when the rotating cylinder makes permanent contact with the surrounding wooden guide-piece.
  12. At the end of 30 seconds, stop the rotator.
  13. Turn on the light of the potash illuminator.
  14. Remove the vial from the rotator and insert into a slot of the illuminator, then move back and forth over the lined chart and at the same time sight down through the column of the test liquid.
- If heaviest lines cannot be seen, reading is 4.
- If heaviest lines can be seen but medium lines are invisible, reading is 3.
- If medium lines can be seen but light green lines are invisible, reading is 2.
- If light green lines can be seen, reading is 1.
- Important:* The solution in the vial must be smooth. If the solution is curdy, or a crystalline precipitate forms, repeat, using less filtrate.
15. If a reading of 1 is obtained, use 0.6, 0.7, 0.8, 0.9 or 1.0 ml. of the filtrate, making up to a full ml. in each case with Reagent 1,  $K_2O$  solvent.
  16. If a reading of 4 is obtained, use 0.4, 0.3, 0.2 or 0.1 ml. of the filtrate, making up to a full ml. in each case with Reagent 1,  $K_2O$  solvent.
  17. Add Reagents 2 and 3,  $K_2O$ , as before, mix in the rotator and take reading in the potash illuminator.
  18. Refer to Table 1 for per cent potash ( $K_2O$ ).
  19. In case a 4 reading is obtained when 0.2 or 0.1 ml. of the filtrate is used, pipette out 10 ml. of the filtrate and add 40 ml. of Reagent 1,  $K_2O$  solvent, to make up to a total volume of 50 ml. Mix thoroughly.
  20. Take 1.0, 0.9, 0.8 ml., etc., of this solution in a comparison vial, make up to a full ml. with Reagent 1,  $K_2O$  solvent, and continue the test as before.
  21. Use Table 2 for potash content of ash.



TABLE FOR RAPID ESTIMATION OF POTASH IN MILL ASH  
(1 gram ash, 50 ml. Reagent 1, K<sub>2</sub>O solvent.)

TABLE 1

Reading	K <sub>2</sub> O content	ml. of filtrate taken										
		1.0 ml.	0.9 ml.	0.8 ml.	0.7 ml.	0.6 ml.	0.5 ml.	0.4 ml.	0.3 ml.	0.25 ml.	0.2 ml.	0.1 ml.
2	Per Cent	.38	.42	.47	.54	.63	.75	.94	1.25	1.5	1.9	3.8
3	Per Cent	.43	.47	.53	.61	.71	.85	1.1	1.4	1.7	2.1	4.3

NOTE: Reading 1 is less than the respective 2 readings.

Reading 4 is greater than the respective 3 readings.

TABLE 2

(1 gram ash, 50 ml. Reagent 1, K<sub>2</sub>O solvent, 10 ml. filtrate plus 40 ml. Reagent 1 K<sub>2</sub>O solvent.)

Reading	K <sub>2</sub> O content	ml. of filtrate taken										
		1.0 ml.	0.9 ml.	0.8 ml.	0.7 ml.	0.6 ml.	0.5 ml.	0.4 ml.	0.3 ml.	0.25 ml.	0.2 ml.	0.1 ml.
2	Per Cent	1.9	2.1	2.3	2.7	3.1	3.8	4.7	6.3	7.5	9.4	19.0
3	Per Cent	2.1	2.4	2.7	3.0	3.5	4.3	5.3	7.1	8.5	10.6	21.0

NOTE: Reading 1 is less than the respective 2 readings.

Reading 4 is greater than the respective 3 readings.

## II. Determination of Phosphoric Acid, $P_2O_5$ in Mill Ash

1. Weigh 1 gram of prepared sample from (G) and transfer to a 125-ml. flask.
  2. Add 50 ml. N/2 HCl to the sample in the flask.
  3. Stir for 3 minutes and filter through a Munktell No. 3, 12.5-cm. filter paper into a 100-ml. beaker.
  4. Transfer 10 ml. of the filtrate by means of a 10-ml. pipette into a 50-ml. volumetric flask and make up to its 50-ml. mark with distilled water.
  5. Mix the solution in the flask several times.
  6. By means of a special 0.5-ml. pipette, transfer a 0.3-ml. portion of the diluted solution (5) to a special phosphate vial.
  7. Then add Reagent 4,  $P_2O_5$ , into the vial up to the mark indicating an 8-ml. volume.
  8. Mix well by inverting tube several times.
  9. Add 1 or 2 drops of stannous chloride and mix again.
  10. Immediately compare the developed color with the same set of color standards employed for the rapid chemical analysis of phosphate in cane juice.
- Note A: In case the color developed is darker than the No. 8 standard tube, take the next smaller portion of filtrate, 0.2 ml., and repeat steps Nos. 9 to 12.
- Note B: Conversely, when the color is too light, use a larger portion of 0.4 ml. or 0.5 ml. and repeat steps Nos. 9 to 12.
11. Refer the number on the standard matched and the number of ml. taken to the data sheet for mill ash analysis and obtain percentages of  $P_2O_5$  in the sample.

TABLE FOR RAPID ESTIMATION OF PHOSPHORIC ACID IN MILL ASH

(Figures represent percentages of  $P_2O_5$  in Ash)

$P_2O_5$ Standard Tube Matched	ml. of Solution Taken for Comparison				
	0.1 ml.	0.2 ml.	0.3 ml.	0.4 ml.	0.5 ml.
1	..	4.0	2.7	2.0	1.6
2	..	6.0	4.0	3.0	2.4
3	..	8.0	5.3	4.0	3.2
4	..	10.0	6.7	5.0	4.0
5	..	12.0	8.0	6.0	4.8
6	..	14.0	9.3	7.0	5.6
7	..	16.0	10.7	8.0	6.4
8	..	18.0	12.0	9.0	7.2

## RAPID ESTIMATION OF CALCIUM IN SOILS

### *Equipment Required*

- 12 Flasks, Erlenmeyer, 125-ml. cap.
- 1 pkg. No. 3 Munktell filter paper, 11 cm.
- 12 Funnels, 65 mm.
- 1 Funnel support.
- 24 Vials, shell, short form.
- 12 Beakers, Pyrex, 50-ml. cap.
- 1 Pipette, Mohr, 2-ml. cap., marked to deliver in 0.25-ml. portions.

- 1 Pipette, medicine dropper, calibrated to 1 ml.
- 1 Spatula, stainless steel, 4-inch blade.
- 1 Calcium illuminator.
- 1 Standard calcium chart.
- 1 Burette, dispensing, 250-ml. cap.
- 1 Cover for 250-ml. dispensing burette.
- 1 Burette, Exax, 50-ml. cap.
- 1 Cover for 50-ml. burette.
- 2 Supports, iron, 6 in. x 9 in.
- 1 Burette clamp, castaloy, large, with rubber-covered jaws.
- 1 Burette clamp, Lincoln.
- 1 Metal soil cup, 2.5-gm. cap.
- 1 gal. Reagent 13, Ca.
- 1 pt. Reagent 14, Ca.

#### *Additional Equipment Recommended*

- 1 Metal soil cup, 5.0-gm. cap.
- 1 Metal soil cup, 10.0-gm. cap.

#### *Equipment Required for Calcium Soil Determinations Not Included in Potash or Phosphate in Soil Ensembles*

- 1 Pipette, Mohr, 2-ml. cap.
- 1 Calcium illuminator.
- 1 Standard calcium chart.
- 1 gal. Reagent 13, Ca.
- 1 pt. Reagent 14, Ca.

#### *Procedure*

1. Fill a 2.5-gram metal cup with prepared soil and level off with a stainless steel spatula. Transfer to a 125-ml. Erlenmeyer flask.
2. Add 30 ml. of Reagent 13, Ca, from a 250-ml. dispensing burette.
3. Shake with a rotating motion for  $\frac{1}{2}$  minute, giving approximately 100 swirls.
4. Immediately filter through Munktell No. 3, 11-cm. filter paper into a 50-ml. beaker.
5. Transfer 1.0 ml. into a comparison tube by means of a 2-ml. Mohr pipette.
6. Add 1.0 ml. of Reagent 13, Ca, from a 50-ml. burette.
7. Add 1.0 ml. of Reagent 14, Ca, from a calibrated medicine dropper.
8. Immediately close the tube with your thumb and shake the tube with sufficient rapidity to allow 30 shakes in less than 7 seconds.
9. Let stand for  $\frac{1}{2}$  minute.
10. With the light turned on, place the tube in the middle slot of the illuminator slide. Close the other 2 slots with your fingers.
11. Move back and forth over the lined chart and at the same time sight down through the column of the test liquid:
  - If heavy lines cannot be seen, reading is 4.
  - If heavy lines can be seen, but medium lines are invisible, reading is 3.

If medium lines can be seen, but light lines are invisible, reading is 2.

If light lines can be seen, reading is 1.

12. Whatever the initial reading, it is necessary to check or amplify the result by repeating steps Nos. 5 to 11 with different aliquots of the filtrate until for each soil sample consecutive readings of both 2 and 3 are obtained. The appropriate lines must be clearly seen before being recorded as 2 or 3. With any aliquot make up the volume to 2 ml. with Reagent 13, Ca, before adding 1 ml. of Reagent 14, Ca. In using a burette to add Reagent 13, Ca, run out enough of the reagent into a flask provided for the purpose so as to start from a full ml. mark.

13. If an initial reading of 4 is obtained, use aliquots of 0.75 ml., 0.50 ml., and 0.25 ml. until readings of 3 and 2 are obtained.

14. If the initial reading is 3, use the aliquots above (step No. 13) until a reading of 2 is obtained.

15. In case of an initial reading of 1, use aliquots of 1.25 ml., 1.50 ml., 1.75 ml., and 2.0 ml. until readings of 2 and 3 are obtained.

16. If the initial reading is 2, use the aliquots above (step No. 15) until a reading of 3 is obtained.

17. If necessary, prepare a fresh extract of 5 grams, 10 grams, 20 grams or 30 grams of soil with 30 ml. of Reagent 13, Ca, and proceed as above until consecutive readings of both 2 and 3 are obtained.

18. With the recorded readings of 2 and 3, refer to the table and estimate the percentage and pounds per acre-foot of CaO by taking the lowest figure represented.

Examples:

(A) 2.5-gram extraction:

Aliquots	Readings	Per cent CaO	Final Result	
			% CaO	lb CaO/a-ft.
1.00	2	0.22	0.22	5500
1.25	3	0.25	(lowest value)	

(B) If a reading of 2 is finally obtained for a 2-ml. aliquot, prepare another extraction with 5 grams of soil to 30 ml. Reagent 13, Ca.

2.5-gram extraction:

Aliquots	Readings	Per cent CaO
1.00	1	<0.22
1.25	1	<0.17
1.50	1	<0.14
1.75	1	<0.12
2.00	2	0.11

5.0-gram extraction:

			Final Result	
Aliquots	Readings	Per cent CaO		
			% CaO	lb CaO/a-ft.
1.00	2	0.11		
1.25	2	0.087	0.087	2175
1.50	3	0.10	(lowest value)	

TABLE FOR CALCIUM IN FILTER CAKE

Aliquot	Reading of 3 Calcium, CaO		Reading of 4 Calcium, CaO	
	Per cent	lb/ton	Per cent	lb/ton
1.50	.24	4.8	.35	7.0
1.40	.26	5.2	.37	7.4
1.30	.28	5.6	.40	8.0
1.20	.30	6.0	.43	8.6
1.10	.33	6.6	.48	9.6
1.00	.36	7.2	.52	10.4
.90	.40	8.0	.58	11.6
.80	.45	9.0	.65	13.0
.70	.51	10.2	.74	14.8
.60	.60	12.0	.87	17.4
.50	.72	14.4	1.04	20.8
.45	.80	16.0	1.16	23.2
.40	.90	18.0	1.30	26.0
.35	1.03	20.6	1.49	29.8
.30	1.20	24.0	1.73	34.6
.25	1.44	28.8	2.08	41.6
.20	1.80	36.0	2.60	52.0
.15	2.40	48.0	3.47	69.4
.10	3.60	72.0	5.20	104.0

## ESTIMATION OF SOIL REACTION (pH)

*Equipment Required*

- 1 Soil reaction color chart.
- 2 Porcelain spot plates.
- 3 Porcelain test plates, triple channel.
- 6 Glass rods with fine points.
- 1 Spoon, horn, small.
- 1 Spatula, stainless steel, 4-inch blade.
- 1 Brush, camel hair, pencil, medium size.
- 1 pkg. Munktell No. 3, 9-cm. filter paper.
- 4 Bottles, dropping, with pipettes, 30-ml. cap.
- ½ pt. Bromthymol blue indicator.
- ½ pt. Bromcresol green indicator.
- ½ pt. Phenol red indicator.
- ½ pt. Chlorphenol red indicator.
- ½ pt. Distilled water.

*Equipment Required for Estimation of Soil Reaction Included in  
Soil Potash or Phosphate Assemblies*

- 1 Spatula, stainless steel, 4-inch blade.
- 1 pkg. Munktell No. 3, 9-cm. filter paper.

*Preliminary Test, Using the Spot Plate*

- 1. Using a small spoon or a stainless steel spatula, place prepared soil in a depression of the spot plate until it is about ⅓ full.
- 2. Add bromthymol blue indicator drop by drop until the soil is saturated; add 3 drops more.
- 3. Pick the plate up and shake it for about 10 seconds (12 counts) by a motion which may be described as a combined rocking and rotary motion.

4. Put the plate back on the table and using the large end of the glass rod transfer a drop of the liquid to the flat surface adjoining the depression.

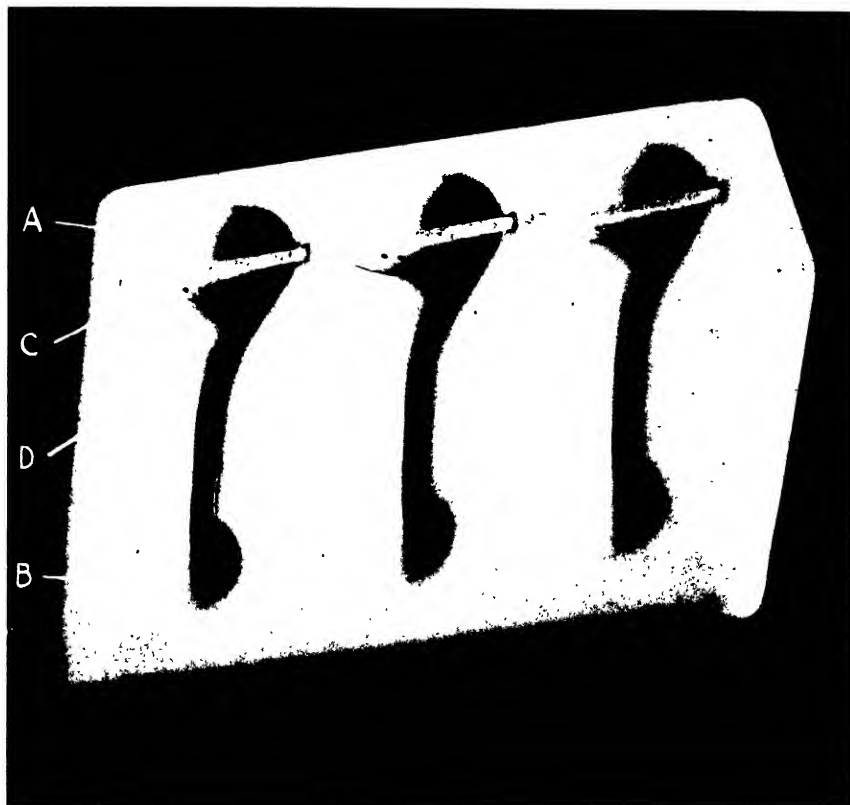
5. Compare the color of the drop with the colors of the bromthymol blue color chart.

6. If a match is not obtained, repeat steps Nos. 1 to 6, using chlorphenol red or phenol red, depending on whether the pH of the soil is below or above the bromthymol blue range, respectively. The test can be further extended on the acid side by using the bromcresol green indicator. If the pH appears to be below 3.8 or above 8.2, bromphenol blue or thymol blue indicators and their respective color charts may be used. These are available and will be supplied upon request.

7. When a match is obtained with any indicator near the mid-portions of the corresponding color chart, proceed with the triple channel block for the final and accurate determinations.

8. If a match is obtained but near the end portions of the chart, the test should be repeated with the indicator next in the series to determine which of the two will give readings closer to the middle portions of the chart before proceeding with the use of the triple-channel block.

*Procedure for Use of Triple-Channel Block*



A—Depression  
B—Reservoir  
C—Partition  
D—Channel

1. By means of a small horn spoon or stainless steel spatula, fill with soil the 3 depressions marked "A" on the drawing. Press the soil lightly against the partition "C" with the spoon or spatula to make certain that the soil is in complete contact with the partition.

2. Using a camel hair brush, remove completely any soil that may have fallen into channel "D" or reservoir "B."

3. Add the indicator, predetermined by the preliminary test, drop by drop until the soil is saturated; add 2 or 3 additional drops.

4. Apply the sharp end of the glass rod very lightly against the minute openings of the partition "C" on the side opposite the soil, and gently draw the solution out into channel "D." With the large end of the glass rod gently draw a small portion of the solution into reservoir "B."

*Precaution:* Draw the liquid into the channel and reservoir; *do not* rub the partition or the surface of the porcelain block with the glass rod.

5. Immediately compare the color of the liquid in depression "B" with the color chart of the respective indicator used.

Note: In case the color of the liquid in reservoir "B" appears deeper in intensity than those of the chart, transfer a drop of the solution by means of the glass rod to the flat surface adjacent to the reservoir and compare the color of this drop with the colors of the chart. When the liquid comes out too pale in color, as often happens, with the bromthymol blue indicator, wipe off the liquid with a sheet of clean filter paper and draw out more solution, applying more indicator to the soil if necessary.

6. When a match is obtained near the mid-portions of the chart (for example: 6.4 to 7.2 on the bromthymol blue chart) record the reading. Determinations should at least be made in triplicate, two of which should check before reporting the result.

7. If a match is obtained, but near the end portions of a chart, make a few additional determinations using the indicator next in the series. When the results obtained by the two indicators do not agree, report the average of the two results as the pH of the soil.

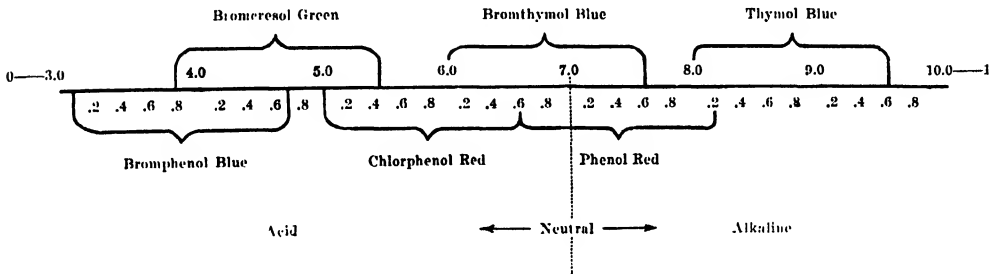
8. Interpretation of results: A reaction of pH 7.0 indicates a neutral soil, that is, one which is neither acid nor alkaline. Values below pH 7.0 denote acidity, the intensity of acidity increasing as the numbers decrease. Values above 7.0 denote alkalinity, the intensity of alkalinity increasing as the numbers increase.

### Precautions

1. The color chart should be kept in the envelope provided for the purpose when not in use since exposure to light accelerates fading of the colors.
2. Make readings in a bright, natural, diffused daylight. Readings made in dazzling sunlight or in artificial light may be incorrect.
3. Do not conduct the test while ammonia or acid fumes are present in the laboratory.

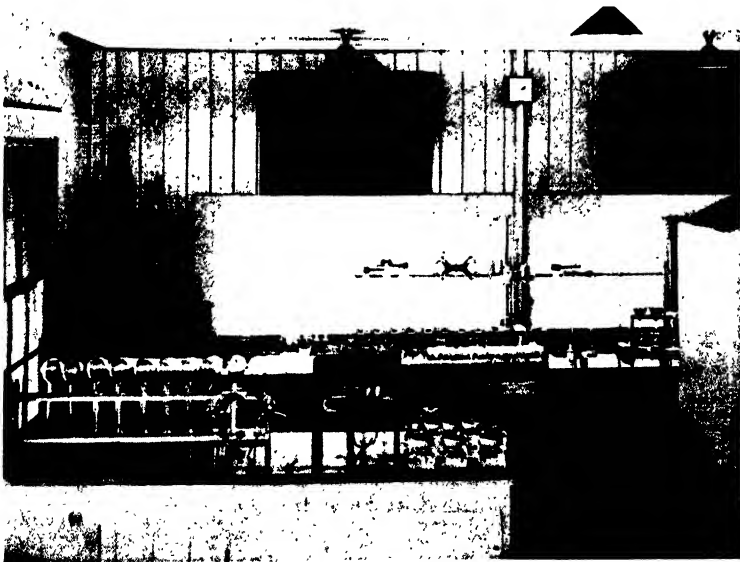
### SOIL REACTION (pH)

Indicator Series:



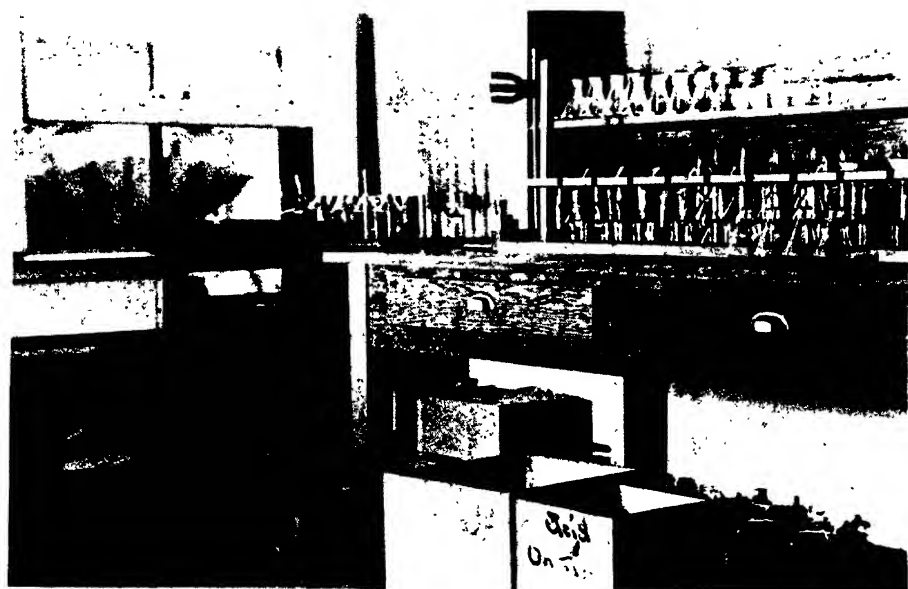
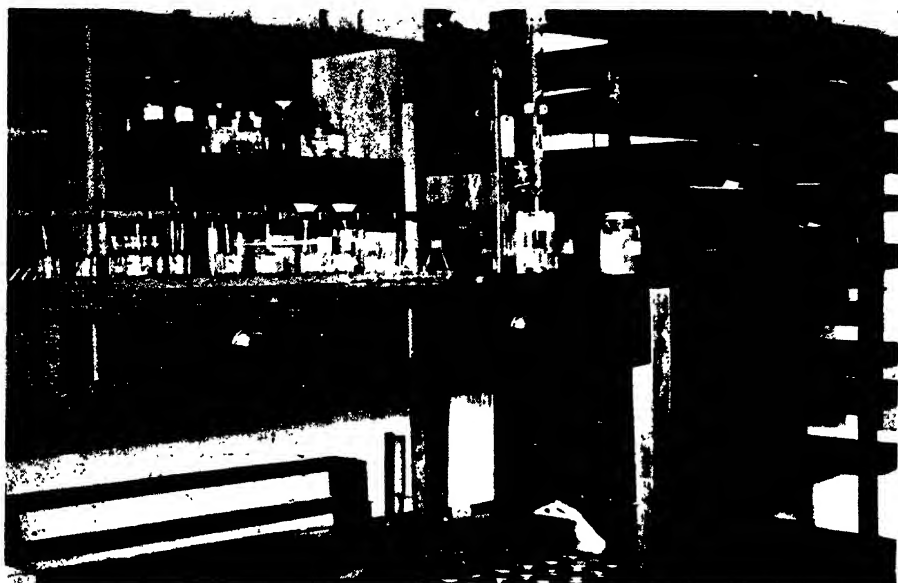
### PLANTATION R. C. M. LABORATORIES

The following illustrations depict views of some of the plantation R. C. M. laboratories.

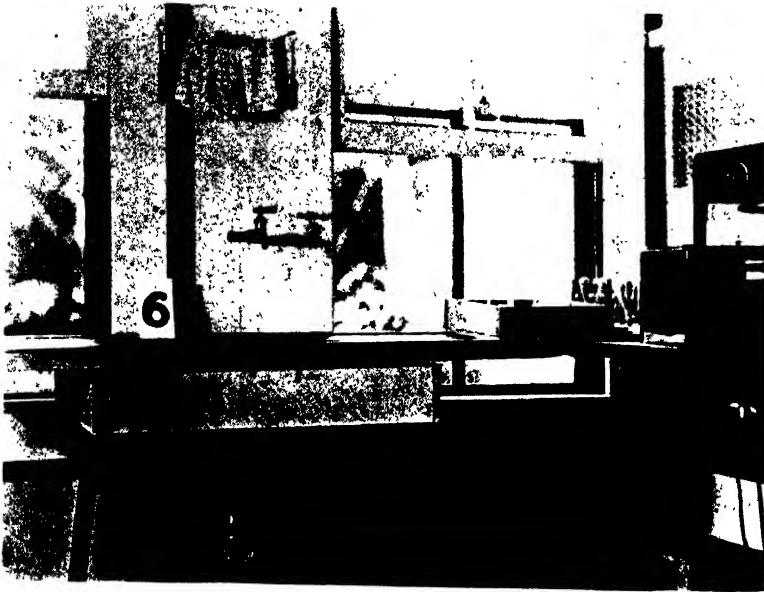


A view of the R.C.M. laboratory at Pioneer Mill Company, Ltd.

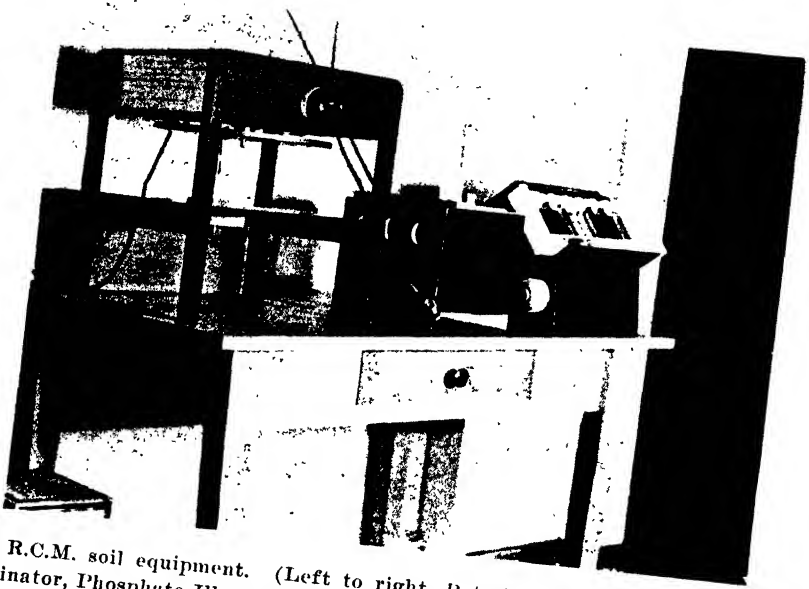




Views of R.C.M. laboratory of island representative, Experiment Station, H.S.P.A. (Maui).



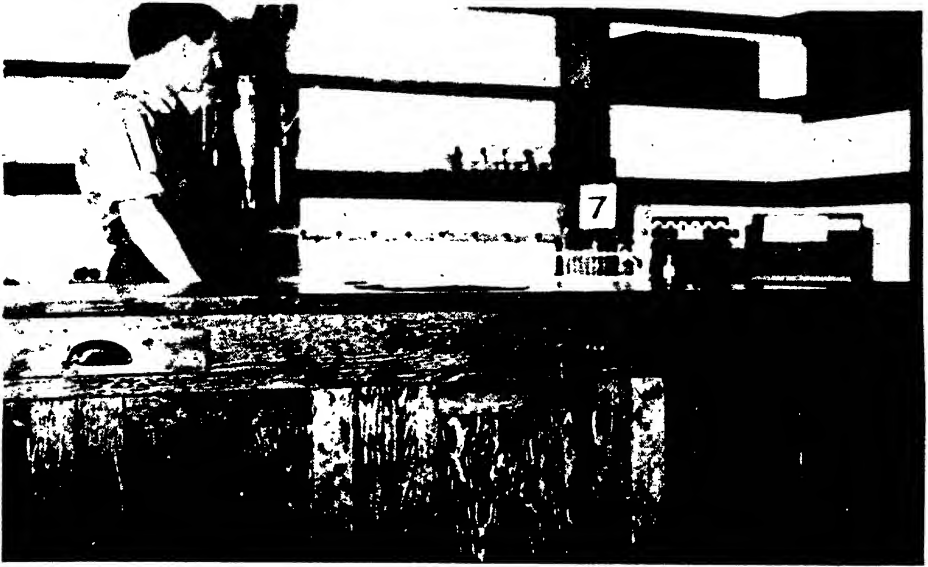
Laboratory views, island representative, Experiment Station, H.S.P.A. (Maui).



R.C.M. soil equipment. (Left to right, Potash Rotator, Potash Illuminator, Phosphate Illuminator.)



Preparing soils for analysis.



A view of the Waiakea Mill Company R.C.M. laboratory



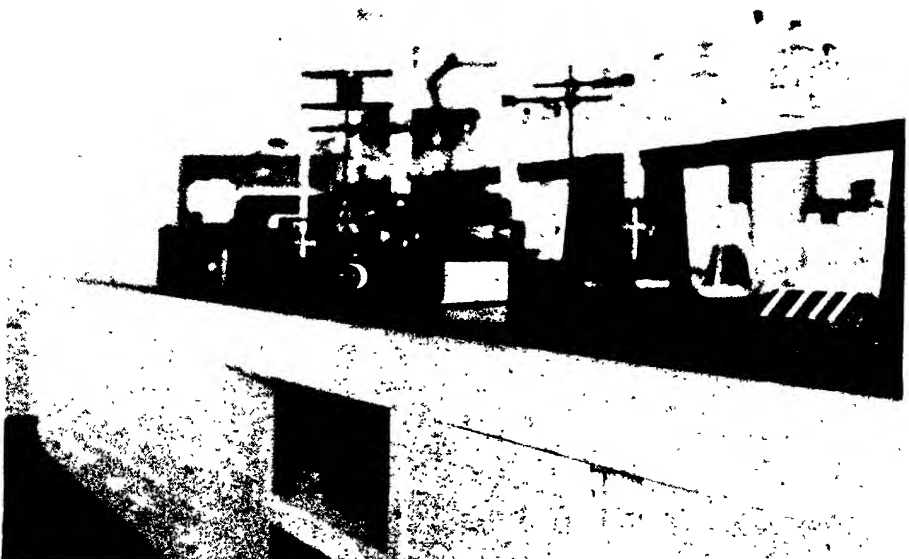
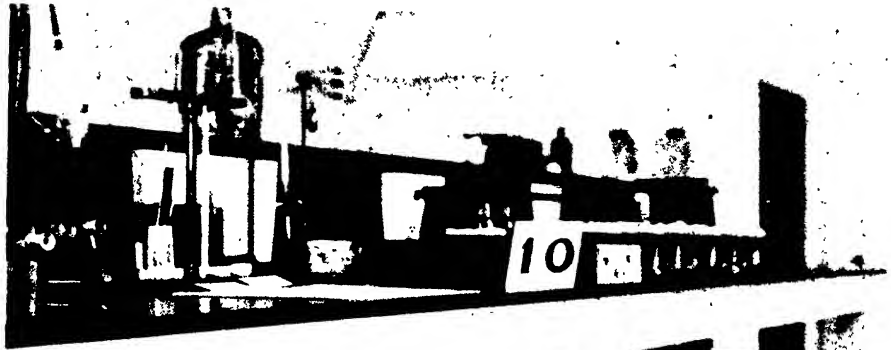
A corner of Olaa Sugar Company, Ltd., R.C.M. laboratory.



One end of the R.C.M. laboratory at Hutchinson Sugar Plantation Company.



An outside view of the Hutchinson Sugar Plantation Company R.C.M. laboratory.



Two views of the R.C.M. laboratory at Hawaiian Agricultural Company.



View of the R.C.M. laboratory at Kilauea Sugar Plantation Company.



A corner of the R.C.M. laboratory at Paauhau Sugar Plantation Company.



A portion of the R.C.M. laboratory of the island representative, H.S.P.A. (Hilo).

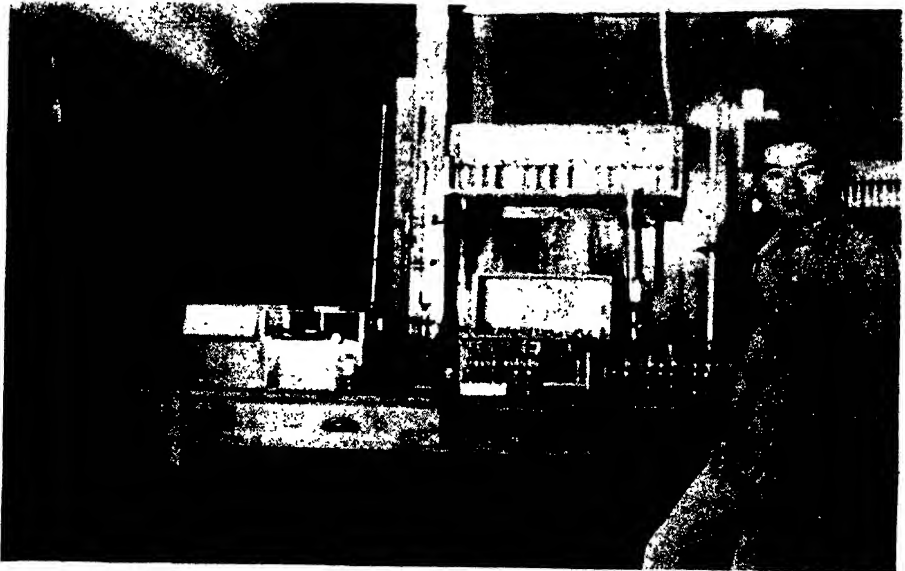


A view of the R.C.M. laboratory at Hakalau Plantation Company.

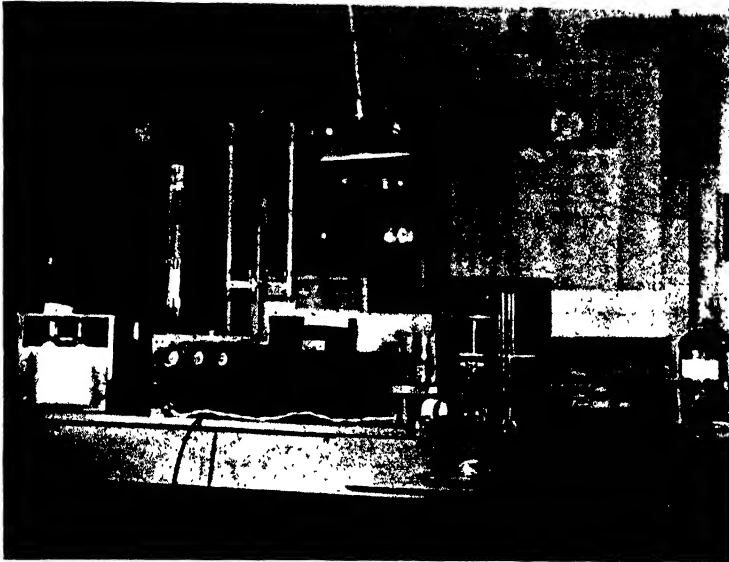




A corner of the R.C.M. laboratory at Honomu Sugar Company.



One end of the R.C.M. laboratory at The Lihue Plantation Company, Ltd.



The R.C.M. laboratory of the island representative of Experiment Station, H.S.P.A. (Kauai).

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## Sugar Prices

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96° CENTRIFUGALS FOR THE PERIOD  
MARCH 23, 1936, TO JUNE 3, 1936.

Date	Per Pound	Per Ton	Remarks
March 23, 1936.....	3.60¢	\$72.00	Puerto Ricos.
“ 30 .....	3.75	75.00	Puerto Ricos.
May 4 .....	3.72	74.40	Puerto Ricos.
June 3 .....	3.76	75 20	Cubas.

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# THE HAWAIIAN PLANTERS' RECORD

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No. 4

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## **In This Issue:**

### *A Giant Macadamia Nut:*

A species of macadamia nut, over twice the size of the varieties now growing in Hawaii, was discovered in the forests of North Queensland in 1889. Plans for its importation into Hawaii are now underway by the Botany and Forestry Department. A single tree was located in August 1935, near the point of original discovery, from which it is hoped viable seed may be obtained. A recent expedition in search of it is described, together with facts pertaining to its history, usefulness and normal habitat.

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### *A Preliminary Report on an Entomological Survey of Guam:*

Transpacific airplane service opens a new gate for the entrance of foreign insect pests to our Territory. The island of Guam is the most important and largest stopping place for planes plying between the Philippines and Hawaii and a stepping stone for oriental pests eastward. An entomological survey of that Island by O. H. Swezey is placing on record vital information of great value in the formulation of quarantine procedures for Hawaii and incidentally for the mainland of the United States.

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### *Comparative Hardness of Tasseled versus Untasseled Canes:*

In 4500 tests comparing the rind hardness of tasseled versus untasseled canes all of the same age and growing in close proximity, the former are shown to be consistently softer. These comparative studies were made possible in the field through the use of an instrument specially designed for the purpose. This is described and illustrated. The bearing of this investigation on beetle borer infestations is discussed.

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### *Soil Reaction and Total Acidity:*

The meaning and significance of such terms as reaction, pH, total acidity and buffers are discussed, with special reference to Hawaiian soils. The processes by which Hawaiian soils have been formed and have become acid or alkaline are de-

scribed. The effects of various agricultural practices upon soil reaction and total acidity and measures to prevent undesirable effects due to excessive acidity or alkalinity are outlined.

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*The Fluctuations of Sugars in the Leaf Sheaths of the Sugar Cane Plant  
During the Day and the Night:*

Problems concerning the movement of sugar from the blade to the stem of the sugar cane plant are presented, the discussion being based upon analyses of sheaths taken at hourly intervals during the day and the night. The results indicate that the transport of sugar occurs both day and night, that both cane sugar and simple sugars are translocated, and that the factors affecting the movement of sugar include the amount of water and interchanges with other compounds.

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*Further Notes on Water and Cane Ripening:*

This is the second in a series of studies designed to determine what effects the withholding or supplying of water will have upon the formation and storage of sugar in the cane plant. The results indicate that the plants receiving water were superior in the manufacture of sugar in the leaves and in its transport to the stems, whereas the plants deprived of water were superior in the storage or conservation of sugar in the stems. Although the plants deprived of water had the higher percentages of cane sugar in the tissue of the stem, the plants receiving water yielded, in general, the better juices, indicating that the sugar present in the plants receiving water was more readily expressed than that in the plants deprived of water.

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## A Giant Macadamia Nut

By C. E. PEMBERTON

In 1889, F. Manson Bailey, Colonial Botanist of Queensland, Australia, while engaged in a botanical exploration of the dense tropical forests of the cloud-enshrouded Bellenden Ker Range in North Queensland, which composes the highest mountains of that state, discovered a new species of macadamia nut, the fruit of which measured over two inches in diameter. In a comprehensive botanical publication (*The Queensland Flora*, part 4, page 1330. 1901), appears Bailey's description of this particular tree, under the name *Macadamia Whelani* Bail., together with the statement that it was "Abundant along Tringilburra Creek, and thence to Whelanian Pools," and further that "The nuts seem to be largely used by the natives of this locality for food, as we found large quantities of the broken shells as well as the whole nuts at all their camps." Beyond these original observations by Bailey and his final, brief discussion of it in the above monograph in the terse, technical language of the thorough taxonomist, apparently no further notice was given to the existence of this interesting tree until Dr. H. L. Lyon gave it consideration as a species desirable for study and introduction to Hawaii.

During May 1935, R. W. Mungomery, Chief Entomologist for the Queensland Bureau of Sugar Experiment Stations, spent some weeks visiting at the Experiment Station of the H. S. P. A. and when questioned by Dr. Lyon as to the location of Tringilburra Creek, above mentioned, indicated its position on the map. Later in the year when the writer was in Queensland near the Bellender Ker mountain range with Mr. Mungomery, the creek was pointed out where it could be seen descending from a densely forested mountain region into the valley below. Decision was immediately made to penetrate the range up this creek in an effort to re-discover this large-fruited macadamia nut. The exit of this creek into the lowlands was found to be so choked with high, impenetrable thickets of lantana, greatly exceeding those which may be found anywhere in Hawaii today, that Fishery Falls Creek, some 5 miles away, which led out from the same mountain range, was selected for examination because more easily approached and provided with a well-cut trail. This creek lies some 24 miles from Cairns, North Queensland and tumbles directly out of the dark Bellenden Ker Range as a cool, clear stream of fair volume.

On September 18, 1935, the writer, accompanied by J. H. Buzacott, Assistant Entomologist, Queensland Bureau of Sugar Experiment Stations, visited Fishery Falls Creek in search of the tree in question. After following the creek bed and carefully examining the trees for an hour or more, until the fine, thick forest, which completely clothes the mountain, had been well entered and the stream bed had become steep and more difficult of passage, a halt was made in the deep shade among huge boulders and a rushing torrent. No signs of the coveted tree had been seen and it was feared the search was in vain, when a single, very large and completely hollowed out macadamia nut, half eaten by rats, was discovered lodged in a crevice between some large boulders. By radiating from this spot and carefully searching amongst the leaf mould and rocks near the stream, several more nuts in a similar

condition were picked up. Ultimately a fine large macadamia tree was found some two feet in diameter near the base, with a smooth, straight trunk extending up 40 feet or more to the first large branch, and with the topmost branches reaching into the sunlight 60 to 70 feet above the floor of the forest. High overhead amongst the upper foliage some branches could be seen bearing a number of apparently ripe nuts and beneath the tree many could be found that had been partially destroyed by rats.

On the following day the party returned to the spot accompanied by G. L. Windred, Chief Entomologist of the Colonial Sugar Refining Company of Australia, who is shown on the cover page of this issue holding a fruiting branch of *Macadamia Whelani*. Ascent of the tree was a difficulty surmounted by Mr. Buzacott, who succeeded in climbing the slippery trunk with the aid of climbing irons attached to his feet, and after much labor obtained most of the fruits on the tree, comprising about 100 in all. These were carefully dried and brought by the writer to Honolulu on October 28, 1935. They were planted under Dr. Lyon's supervision but failed to germinate, which suggests that they were still too immature for removal from the tree when collected, or that the method of transportation was wrong. Further attempts to secure better seed are underway, utilizing again the generous assistance of Messrs. Mungomery and Buzacott, whose help and that of Mr. Windred are hereby gratefully acknowledged.

This tree is of special interest owing to the large size of the nuts. It is rather surprising that attempts to commercialize the species have not been made in view of the findings of Bailey in 1889, who obtained fairly reliable evidence that the nut was edible and in considerable use amongst the natives. In the present instance none of the fruits were eaten. The number collected was so small that all were saved for planting. The shell is very hard and somewhat thicker than that of the common commercially planted macadamia nut, but this nut will probably contain twice as much meat as the smaller species, or perhaps more. It is also interesting to speculate on the possibilities arising from hybridization of the two species.

The accompanying photographs will serve to illustrate the tree from which the nuts were collected, the size of the fruit, and the general complex of vegetation, all striving for existence in the tangled jungle where the tree was found.



Fig. 1. The lower portion of the trunk of *Macadamia Whelani* Bail. Diameter about  $1\frac{1}{2}$  to 2 feet.





Fig. 2. Showing a well grown specimen of *Macadamia Whelani* Bail. at left center, in a typical North Queensland tropical forest. The figure of a man can be seen climbing the tree half way up the trunk, which will indicate the proportions of the tree.

## A Preliminary Report on an Entomological Survey of Guam\*

By O. H. SWEZEY

We arrived in Guam on April 27, 1936, and commenced the survey as soon as possible after getting settled in a residence, provided by the Governor, on the grounds of the Edmund S. Root Agricultural School at Piti, 4½ miles southwest of Agana, the Capital city. An endeavor has been made to ascertain all the insects connected with each of the various crops. The small farms, or ranches (no matter how small, they are called ranches, and a few are as extensive as two or three thousand acres, much of which is wild land) are scattered over all parts of the Island. In some regions the land is fully occupied agriculturally, but many ranches are in clearings in the midst of dense tropical jungle. Many of the ranches have been visited in various sections in order to make observations on the insects associated with the various crops. Those situated in the clearings in the jungle have made a convenient access to the native trees, and much knowledge has also been gained of the insect faunas of the more important trees. The crop pests, that is, those known at the present time, are listed as follows, names being used when known, but some are as yet without names:

### COCONUT

The coconut is the only crop being produced for export; 1809 tons of copra being exported last year. Coconut trees are everywhere from one end of the Island to the other. Most of them have been planted, but some are volunteers. Much of the corn and vegetable crops are grown beneath or among the coconut trees. At present, coconut trees are not severely injured by insects, but the following attack them to some extent:

*Aspidiotus destructor* is the notorious coconut scale which so nearly ruined the coconut trees of Saipan some years ago. It never became so serious on Guam, as it has been controlled by a tiny black ladybeetle (*Cryptogonus nigripennis*) and a few chalcid parasites, the ladybeetle being the most important. At present, only an occasional small colony of the scale is to be found on the coconut leaves. It is likewise occasionally found on banana leaves, and I have also found it on avocado, mango, grape, rose, and jasmine.

*Pseudococcus cocotis* is a mealybug found to some slight extent on coconut trees.

Phasmids: One or possibly two species of stick insects sometimes feed extensively on the leaves, giving them a somewhat ragged appearance.

Tineid: The larvae of a small moth feed on the under side of leaves, singly, and produce short, narrow, dead streaks where they eat off the under part of the leaf. From the present appearance of the leaves, these larvae must have been very numerous at some time, but during the months I have been here, they have been very scarce.

\* Printed with the kind consent of Governor B. V. McCandlish of Guam, under whose jurisdiction this survey is being conducted.

*Rhabdocnemis obscura* and *Diocalendra taitense*: The larvae of these two weevils feed to some extent in the stubs of cut off leaves. Once an old cocoon of *obscura* was found in the husk of a coconut where the larva had fed.

*Necrobia rufipes* is of course present, as it is always on copra.

*Scholastes bimaculatus*: The maggots of this fly feed in decaying coconuts; it is not common.

#### CORN

Corn is the next crop of importance. It is grown by all the ranchers, and is largely used for human food, both green and when ripe. Two to three crops are grown annually, and it is found in all stages of growth at all times. Its pests are as follows:

*Pyrausta nubilalis*, the European corn borer, once a pest reported as taking half of the corn crop, is now satisfactorily controlled by a tachinid fly (*Ceromasia lepida*) introduced from Japan in 1931. In the mainland of United States this corn borer feeds on many kinds of plants, but in Guam, I have found it only on corn.

*Chloridea obsoleta*: The corn earworm is the most injurious pest. About 2 per cent or more of the ears are attacked by it, and sometimes the percentage is higher, but I have not found it to be nearly the pest that it is in Hawaii during recent years.

*Marasmia trapezalis*: The corn leafroller is nearly always present on young corn plants, the caterpillars rolling the tip portion of the leaf for protection. If growing conditions are favorable, the plant is not appreciably checked by the leaf-rollers. These leafrollers are less common as the plant becomes older; some are parasitized by a braconid wasp (*Apanteles* sp.).

Agromyzid: A tiny black fly the larvae of which feed very abundantly in the leaves of young corn plants. They produce narrow mines running longitudinally, often as many as one hundred per leaf. They are most numerous in the apical half of the leaf, causing early drying up and dying of the leaf. The succeeding new leaves are attacked less, perhaps on account of an Eulophid parasite which works in fair numbers on the leafminer maggots.

*Prodenia litura*: Caterpillars of this noctuid moth have occasionally been found feeding on corn leaves.

*Peregrinus maidis*: The corn leafhopper is usually to be found, but not in serious infestations. It is attended by a small green bug (*Cyrtorhinus lividipennis*) which apparently has the same relation to it that *Cyrtorhinus mundulus* has to the cane leafhopper in Hawaii.

*Aphis maidis* often causes bad infestations of the tassels when they are about to expand. A large ladybeetle (*Harmonia arcuata*) is especially abundant and with one or two others quickly reduce these infestations. The syrphid fly *Simosyrphus grandicornis* is also common, its maggots feeding on the aphids.

Grasshoppers: Two or three species of grasshoppers feed on corn leaves to some extent.

*Calendra oryzae*: The rice weevil is injurious to stored corn. Often a great deal of corn is destroyed when not properly stored, or not properly treated to kill the weevils.

## RICE

Rice is an important crop, being grown in the river valleys where the land is favorably located for irrigation. Only one crop is grown per year, it being planted in September during the rainy season. It ripens for harvesting during the succeeding dry season. Not enough is grown for local consumption, and a considerable amount is imported from Japan. A large number of insects are found in rice fields, some of them causing considerable loss.

*Leptocorisa* sp: The rice bug is a large insect; it punctures and feeds on the soft growing rice kernels.

Mirids: Two species of leaf bugs.

Cicadellids: Two species of leafhoppers.

Delphacid leafhopper: It is parasitized to a considerable extent by a dryinid.

Grasshoppers: Three or four species, one of which feeds largely on the heads of the rice when the kernels are still soft.

Leafroller: The leaves are often eaten by a small caterpillar which rolls the leaf for protection, yet is well parasitized by a braconid (*Apanteles* sp.).

Tineid: A tiny moth larva is sometimes found in the rice heads.

Pyralid moth: The larvae were found feeding among decaying leaves at the base of rice stools. It is not certain whether or not they sometimes feed on the growing plant.

Armyworms are said to cause considerable damage in the seedling plots, but I have not as yet seen any.

## SUGAR CANE

Sugar cane is of little importance at the present time, less of it being grown than in former times. There are patches of not more than two to three acres, and little attention is given to it. The juice is extracted by crude vertical wooden rollers operated by a sweep drawn by a carabao. Boiling is done in large, open, iron kettles fixed in a concrete furnace. The resulting molasses is either used at a local distillery, or made into peanut candy for local consumption. There is a tendency toward a revival of cane growing with the distribution of seed from the Root Agricultural School farm where several varieties were received from Honolulu early in 1935; this seed is distributed at each time of cutting. The cane insects are for the most part the same species as those in Hawaii.

*Rhabdocnemis obscura*: The cane borer is not very injurious where the patches of cane are well taken care of, but in neglected patches it does considerable damage. At one time the tachinid fly *Ceromasia sphcnophori* was established by colonies from Honolulu, but it has entirely disappeared.

*Perkinsiella thompsoni*: A leafhopper closely related to the cane leafhopper in Hawaii. It is very scarce, and its eggs when found are mostly parasitized by a species of *Paranagrus*.

*Trionymus sacchari*: The pink mealybug is often to be found but is not of much importance. A fungus disease seems to control it rather well.

*Pseudococcus boninsis*: The gray mealybug is also occasionally found. A parasite was reared from it which seems to be *Aphycus terryi* which is also present in Hawaii.

*Pseudococcus brevipes*: The pineapple mealybug is also occasionally found on cane.

. *Aphis sacchari* and the stalk mite: I have not seen these on cane in Guam.

Grasshopper: A large species, which is generally prevalent, sometimes feeds on cane and causes a few ragged leaves.

Cane Rust was found in a few cane patches. This is a disease not yet known in Hawaii. Some other peculiar spottings of leaves have been observed, and preserved specimens have been sent to the Experiment Station for study; typical lesions of brown stripe and leaf freckle were found on certain leaves while two unknown leaf markings were observed on other leaves.

#### TARO

Taro is grown everywhere—the upland varieties. I have not found it damaged by insects, although the following are sometimes present:

*Prodenia litura*: Eggs of this noctuid moth are deposited in clusters on the under side of the leaf. The young larvae eat gregariously for a while then scatter to nibble here and there. Most of them disappear before maturity. I think that probably the yellow-jacket wasps prey on them. This and two other wasps are very abundant and always on the search for caterpillars; they are undoubtedly of great importance in the control of this moth, other caterpillars, and the numerous kinds of leafrollers.

*Megamelus proserpina*: The taro leafhopper is sometimes found, but not abundant enough for noticeable injury.

*Aphis gossypii*: The cotton aphid is sometimes found slightly infesting taro leaves. It is parasitized by a species of *Aphelinus* and preyed upon by syrphid larvae.

Aleyrodid: In one taro patch an aleyrodid was found on some of the leaves.

#### TOBACCO

Tobacco is grown only in occasional small patches.

*Chloridea obsoleta*: Caterpillars of the corn earworm spoil many of the leaves unless handpicking is resorted to.

*Prodenia litura*: Caterpillars of this noctuid moth feed on tobacco to a slight extent.

Grasshoppers: Two or three species damage the leaves to some extent.

Mirid bug: A small species is common on tobacco.

#### BANANA

The banana is another plant which is grown very generally, but in a scattered way—not in mass formation. A few species of insects attack it.

*Aspidiotus destructor*: The coconut scale is occasionally found in small colonies on the leaves, a yellowish area indicating the presence of the colony.

*Prodenia litura*: Caterpillars of this moth do more conspicuous damage to the leaves. The freshly hatched caterpillars feed near where the eggs were located, but

soon scatter, nibbling here and there on the under surface of the leaf. When larger they feed along the midrib thus injuring the leaf so that it soon becomes brown and dead.

**Scarabeid:** A large brown beetle which at times feeds extensively on banana leaves at night time, remaining hidden during the day.

**Grasshopper:** A large grasshopper sometimes feeds on banana leaves.

***Cosmopolites sordidus*:** The widely spread banana borer is present. The adult weevils are always to be found in the base of old decaying banana plants, and the larvae are found in growing stems as well.

***Polytes mellerborgi*:** A smaller weevil often found under similar conditions to the banana borer, the larvae feeding chiefly in the base or corm of the plant.

## ORANGE

Several kinds of citrus fruits are grown, and the same kinds of insects affect all of them.

***Papilio xuthus*:** This swallowtail butterfly is very common. Its caterpillars feed on citrus foliage.

**Leafminer:** The larvae of a minute moth mine the newly growing leaves causing them to be very much crumpled and deformed, and eventually to die prematurely.

***Icerya purchasi*:** The cottony cushion scale is well controlled by *Novius cardinalis*, introduced from Hawaii in 1926.

**Scales:** Two or three species are sometimes found.

**Gummosis:** A disease of the bark which is more destructive than the insect pests. Wood-boring beetles attack the trees which are dying from gummosis.

I have not found fruit flies in citrus or any other cultivated fruits, but a pretty species was reared from a native fruit (*Ochrosia* sp.), and was found to be highly parasitized by a braconid (*Opius* sp.).

## PINEAPPLE

The pineapple is not grown extensively. The only insect pest so far noted on it is the mealybug *Pseudococcus brevipes*. It is usually scarce, but an occasional fruit is seen with a considerable infestation. Apparently it is well controlled by a small black ladybeetle with a reddish spot towards the apex of each elytron. It seems to be a species, near *Scymnus bipunctatus*, present in Hawaii. This ladybeetle works on *Ferrisia virgata* and other mealybugs. *Cryptolaemus montrouzieri* is also very common and helps greatly to control the mealybugs.

## MANGO

Mango trees are sometimes nearly defoliated by a chrysomelid beetle (*Phytorus pinguis*). These beetles also feed on many other kinds of trees. They seem to be seasonal, occurring mostly in the dry season. A few scales are also found on mango: *Ceroplastes floridensis*, *Lepidosaphes* sp., *Aspidiotus destructor*, and the thread scale.

## BEANS

Several kinds of beans are grown generally, and attacked by the following:

**Leafroller:** The larvae of an undetermined pyralid moth feed on the leaves to some extent.

*Archips rosaceana*: The larvae of this tortricid leafroller occasionally feed on bean leaves. It is a general pest, occurring on many plants. I have reared it from the leaves of 19 kinds of trees and plants, some of them being native trees. It is an immigrant insect first reported in 1926.

**Tortricid:** The larvae of another tortricid moth are often found in bean pods, eating the seeds both of pole beans and lima beans. It is a moth very much like the moth the larvae of which destroy Koa seeds and also feed in the pods of *Acacia farnesiana* and some other legumes in Hawaii. The larvae destroy a large proportion of the seeds of *Pithecolobium dulce* and *Adenanthera pavonina* here in Guam. I made a count in 29 pods of the latter and 77 per cent of the seeds were found destroyed.

## CABBAGE

At least three kinds of caterpillars feed on cabbages and related vegetables: *Prodenia litura*, *Hellula undalis*, and an undetermined species. The second one named is the most destructive. The cabbage butterfly is not found in Guam.

## MISCELLANEOUS VEGETABLES

Each kind of vegetable grown has one or more particular pests which will not be enumerated in this preliminary report.

## FOREST INSECTS

Besides the leafminers and leafrollers already mentioned, I have reared a large number of species of insects from the various native forest trees, and other caterpillars have also been reared, producing a large assemblage of moths which have never been known or reported from Guam. Probably all of these when studied will be found to be new species. Each is particularly associated with its own special food plant. A great deal of material in the other Orders of insects has also been obtained.

## CROP PESTS IN GUAM WHICH DO NOT OCCUR IN HAWAII

**Coconut:** *Aspidiotus destructor*. Coconut scale.

*Pseudococcus cocotis*. Mealybug.

Phasmid. Stick insect.

Tineid moth.

**Corn:** *Pyrausta nubilalis*. European corn borer.

*Marasmia trapezalis*. Leafroller.

Agromyzid. Leafminer.

- Rice: *Leptocorisa* sp. Rice bug.  
 Mirid. Leaf bug.  
 Cicadellid leafhoppers. Two species.  
 Delphacid leafhopper.  
 Grasshoppers. Three or four species.  
 Leafroller. Pyralid moth.  
 Tineid moth. Larvae in heads.
- Cane: *Perkinsiella thompsoni*. Leafhopper.  
 Cane rust. Disease of the leaves.
- Taro: *Prodenia litura*. Noctuid moth.  
 Aleyrodid.  
*Megamelus proserpina*. Leafhopper.  
 (I recently learned that this has appeared in Honolulu.)
- Tobacco: *Prodenia litura*.  
 Mirid bug.  
 Grasshoppers.
- Banana: *Prodenia litura*.  
*Aspidiotus destructor*. Coconut scale.  
 Scarabeid. Large brown beetle.  
*Cosmopolites sordidus*. Weevil borer.
- Orange: *Papilio xuthus*. Swallowtail butterfly.  
 Leafminer. Minute moth.  
 Gummosis. A bad bark disease.
- Mango: *Phytorus pinguis*. Chrysomelid beetle.  
*Ceroplastes floridensis*. Wax scale.
- Beans: Leafminer. Small moth.  
*Archips rosaceana*. Tortricid leafroller.  
 Pyralid leafroller.  
 Tortricid. Larva in pods.

There are over 30, and about a dozen of them not previously reported from Guam.

#### MOSQUITOES

Two kinds of mosquitoes occur in Guam:

*Culex quinquefasciata* the common night mosquito, which is found breeding in water barrels (gasoline drums), tin cans, pools, hog wallows, etc.

*Aedes* sp. A day mosquito which has not been positively determined yet. It inhabits for the most part the wild lands and forests, where it continually annoys the visitor to those regions. Its bite is not so severe as the bite of the day mosquito in Hawaii. The only place in which I have found their larvae is in the accumulated water at the axils of *Pandanus* leaves. *Pandanus* trees are numerous in the forests, two or three species, and even in dry weather I found that they contained water and mosquito wrigglers.



## HOUSE FLY

House flies are very abundant about houses, and also out in the forests, in fact everywhere. Generally they seem to be a smaller species than the usual house fly. They breed abundantly in cow dung and carabao dung of which there is an abundance for the purpose. Several other flies breed in the same material.

## ACKNOWLEDGMENT

Our work has been facilitated by members of the Governor's office, the Department of Agriculture, and the Edmund S. Root Agricultural School, who have co-operated in rendering assistance in meeting ranchers of the various districts, and in visiting desirable outlying locations in extreme parts of the Island. Captain Stephenson of the Marine Corps supplied us with large-scale contour maps which have been indispensable.

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## Comparative Hardness of Tasseled Versus Untasseled Canes

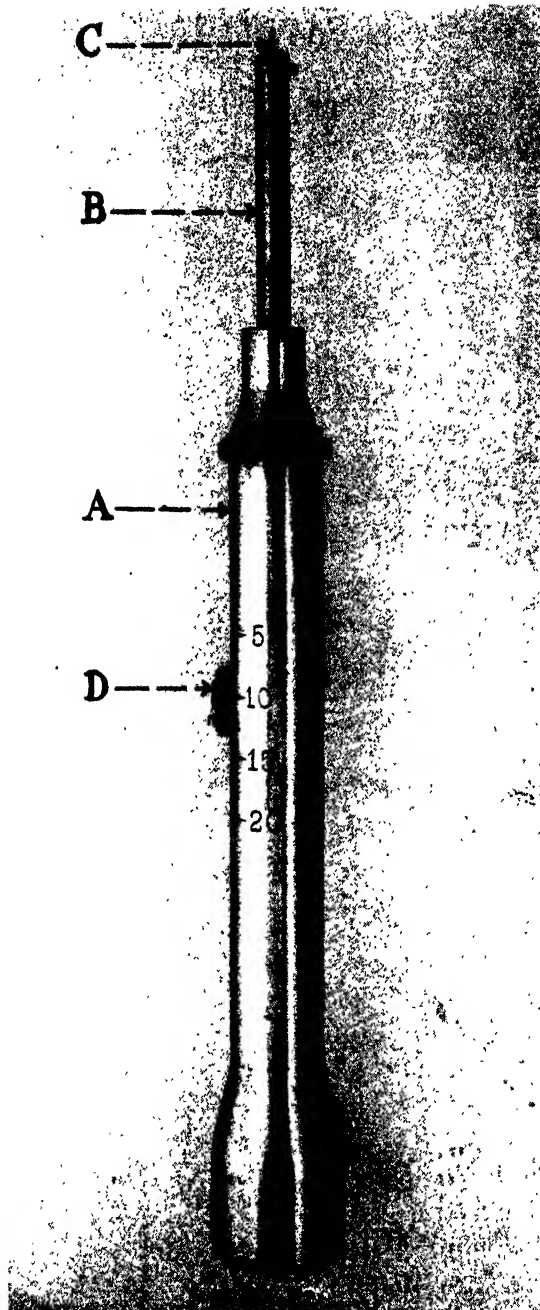
By C. E. PEMBERTON

Soft canes are usually considered susceptible to attack by the beetle borer *Rhabdocnemis obscura* Boisd., while hard varieties are generally believed to be resistant or but slightly damaged by this insect. Actual field experience supports these views. Just why a soft cane offers greater susceptibility than a hard one is problematical, since soft, tender, stalk tissue beneath a live leaf sheath is always accessible to the egg-laying female in even the hardest of canes and is the normal place for the beetle to insert its eggs. The fact, however, that soft canes are more frequently damaged by the beetle borer than hard ones, is so well established that systematic measurements are being made, with special instruments constructed for the purpose, of the hardness of many of the promising seedlings recently produced at this Experiment Station, in the belief that these data will indicate to a certain extent the degree of borer susceptibility to be manifested by each variety when spread on plantations where the borer is prevalent.

A large number of these measurements were made during March and April, 1936. All of the canes had passed through the tasseling season of 1935 and many had tasseled. The tests were made with a new instrument (see accompanying illustration), designed by Dr. H. L. Lyon, which permits field, rather than laboratory study. This instrument is quite similar to one used in India, which was described and illustrated in "Agriculture and Live-stock in India," March 1935, pages 156-158. This greatly facilitated a better interpretation of the data, since the specific environment and growth condition of particular stalks, exhibiting wide variations from the normal of the variety being tested, could be examined on the ground at the time the measurements were being made. In the course of this work, an apparently wide error or variation in the hardness of certain stalks in any given variety was frequently encountered. It soon became obvious that these large deviations from the normal occurred in canes which had tasseled. They were invariably softer than the untasseled canes of approximately the same age, even when both were part of the same stool. This led to a particular study of the relative hardness of tasseled versus untasseled canes in 9 varieties which had tasseled considerably during the latter part of 1935. The varieties selected were chosen at random, with no previous knowledge of their rind characteristics. The results are tabulated below:

TABLE I  
Comparative Hardness of Tasseled Versus Untasseled Canes

Variety	Tasseled		Untasseled		Ratio
	No. Tests	Hardness	No. Tests	Hardness	
POJ 2878 .....	250	4.9	250	10.6	2.16
31-3040 .....	250	7.5	250	14.5	1.93
32-177 .....	250	7.9	250	13.9	1.76
31-3012 .....	250	6.2	250	12.3	1.98
29-2196 .....	250	6.9	250	15.1	2.18
32-1577 .....	250	6.9	250	16.3	2.36
32-1594 .....	250	8.7	250	17.0	1.95
32-1756 .....	250	5.9	250	10.3	1.74
27-8101 .....	250	3.9	250	8.4	2.15



Instrument for measuring the comparative hardness of various canes. Consists of hollow cylinder "A," containing spring attached to plunger "B," which bears removable needle-point "C." Pressure of the point against the cane rind compresses the spring and causes the pointer "D" to slide along the indicator scale and register the number of units of pressure required to force the point into the rind. Reduced one half.

In all of the above tests, both tasseled and untasseled canes were growing in the same field and were approximately the same age. All records were taken in the bottom 4 or 5 joints. No readings were recorded for canes which had tasseled and failed to *lala*, since these stalks were either dead or in the rapid process of dying. Such canes are always very soft.

It will be noted that in 7 out of the 9 varieties selected for study, the untasseled stalks were nearly 2 to 2½ times as hard as those which had tasseled, and in the remaining two cases the untasseled canes were also much harder than those which had arrowed.

During these investigations it soon became evident that stalks in any given variety which *lala* profusely after tasseling, are not as soft as those of the same variety which *lala* very weakly. The process of dying or disintegration after flowering becomes greatly suspended in all parts of the stalk if side shoots are vigorous. This is revealed in the rind studies and can be expressed to some extent in terms of rind hardness, when comparisons are made with untasseled stalks of the same variety, the same age, and from the same part of the field.

The fact that canes which have tasseled are definitely softer than those which have not, may explain the greater incidence of beetle borer infestation in some areas in Hawaii where certain varieties tassel very heavily. Kahuku Plantation Company is a good example to illustrate this point. There tasseling is usually considered excessive with some varieties and beetle borer infestation is often conspicuous. This is particularly to be expected if rainy and windy weather has prevailed after the tasseling season.

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## Soil Reaction and Total Acidity

(A Discussion of Soil pH, Base Exchange and Kindred Topics—  
Terms of Everyday Usage)

By FRANCIS E. HANCE AND L. E. DAVIS

The agricultural importance of "sour" or acid soils has been recognized for a very long time. It was known three thousand years ago that some infertile soils could be improved by liming (16)\*. During the last century the attention of agriculturists was directed more and more toward means for measuring the acidity or alkalinity of soils and correcting adverse soil conditions resulting from excesses of either.

Soil acidity has two quite different aspects which are commonly known as soil reaction and total acidity. The former has been given a great deal of attention by soil chemists who have devised numerous methods and types of apparatus to measure it. Total acidity has been studied somewhat less intensively perhaps; at least routine laboratories usually determine soil reaction more frequently than total acidity.

### ACIDS AND BASES (23)

Before we consider soil acidity it may be desirable to discuss as briefly as possible the meaning of the terms "acid" and "base," as they are used in chemistry. Everyone is familiar in daily life with acids and alkaline substances. Strong *acids* are substances which taste sour, such as the muriatic acid of industry or the citric acid of lemons. They corrode certain metals such as iron and zinc, forming salts of these metals and causing evolution of an inflammable gas—hydrogen. All acids, when in solution in water, yield free hydrogen ions. The chemist classifies as acids many substances which neither taste appreciably sour nor corrode metals, such as the druggists' boracic acid, the soap makers' stearic acid, etc.

Another group of substances commonly known as lyes, caustics, etc., and exemplified by soda lye, borax and ammonia water, is given the name *bases* by chemists. These compounds, when strongly active, combine with fats to produce soaps and are very caustic or corrosive. When a drop of a solution of caustic soda is rubbed between the fingers a sensation of slipperiness is observed which persists to some extent even after washing with water. The weaker members of this family also grade down in intensity of these properties to substances which do not perceptibly exhibit these characteristics. Acids are generally said to have acid or acidic properties; bases, alkaline or basic properties.

Either strongly acid or alkaline substances, when sufficiently concentrated, attack the tissues of plants and animals and are dangerous poisons when taken internally.

\*Numbers in parentheses refer to literature cited. A number of text and reference books dealing with broad phases of the subjects discussed in this paper are included. References to these books may be found immediately after the various sub-titles.

The antidote for an acid is a base, and vice-versa. These two classes of compounds have the property of mutually destroying the primary characteristics of each other. They *neutralize* one another. We thus arrive at a simple and inclusive definition of acids and bases: Strong acids (or bases) may be defined in terms of their most obvious properties; sourness, etc. in the case of acids, or ability to combine with fats to produce soaps, etc. in the case of alkalies; bases (weak or strong) are compounds which neutralize acids; acids (weak or strong) neutralize bases.

#### TOTAL ACIDITY OF SOILS (4, 10, 18)

Soils are exceedingly complex mixtures of many different materials, including fragments of the original rocks from which the soil was formed, mineral constituents formed from the original rocks by weathering, and organic matter produced by the partial decomposition of plant and animal residues. The last two classes are composed of chemically active substances which constitute the seat of the more rapid changes taking place in the soil. These substances may display acid or alkaline characteristics, and the soil is then described as acid or alkaline. The degree of acidity or alkalinity is usually low. Even the most acid soils are by comparison with such substances as vinegar and lemon juice actually only weakly acidified so that an acid soil rarely has a sour taste and does not corrode metals because of its acid constituents alone. However, soils will neutralize and be neutralized by acids or bases if they are alkaline or acidic, respectively. A given quantity of an acid (whether in a solution, soil, or other material) is always exactly neutralized by some definite quantity of an alkaline compound. The amount of the latter required is then a measure of the amount of acid present. This quantity is called the *total acidity*, or (because the determination of its value is frequently made by an analytical procedure called titration) the *titratable acidity*.

As previously stated, when certain metals are attacked by strong acids the gas hydrogen is evolved. It has been established that all solutions of acids contain hydrogen. The distinctive properties of acids are due to hydrogen, although hydrogen in combination with other elements does not always produce acidity. The loss of hydrogen as a gas causes the acidic properties to decrease in intensity and the metals are therefore called bases. Alkaline substances contain metals or certain groups of chemical elements (such as ammonium, a group composed of nitrogen and hydrogen) which play the same rôle as the metals. These metals or allied groups are also called bases.

The acidic properties of soils are due to hydrogen present as a constituent of compounds in the soil. Metallic or other bases are also found in the soil. The acidity or alkalinity of a soil depends upon the relation between the amounts of soil hydrogen and soil bases.

When soluble solid substances, such as common salt, are mixed with water they dissolve and are active chemically. Insoluble substances, such as quartz sand, do not form a solution and are not active. There are many substances which form an intermediate class, of which members of the group of minerals known as bentonites are examples. These materials do not go into true solution; nevertheless they are chemically active. Part, at least, of the hydrogen and bases in these compounds

is present in a condition of incipient solubility. Because of this activity, hydrogen or bases may be replaced by other bases or hydrogen. In the laboratory, replacement is effected by treating the material with a solution rich in the bases (or hydrogen) which are to replace those originally present (8). This phenomenon is called *base exchange* (replacement); the base-exchange (replacement) capacity is the sum of the exchangeable (replaceable) bases and the exchangeable (replaceable) hydrogen. Replaceable hydrogen is generally considered equivalent to total acidity. However, as we shall see presently, there are certain limitations upon this conception.

The chemically active portion of the soil exhibits these properties and is the seat of base exchange. It is sometimes called the base-exchange complex. Since chemical activity is not confined to the laboratory, base exchange is a process which continually operates in nature and as far as agricultural lands are concerned is affected by the addition of fertilizers and soil amendments.

### REACTION AND pH (2, 3, 15)

A portion of the hydrogen of an acid in solution appears to be more reactive than the remainder of the total (acid-forming) hydrogen. The direction and intensity of chemical activity is dependent upon this reactive hydrogen rather than upon the total acidity. The amount of this reactive hydrogen is called the *reaction* and may be very roughly estimated by the degree of sourness. The chemist has more precise means for measuring reaction, among which are the hydrogen electrode, glass electrode, and certain color changes of dyes called indicators.

The reaction of any acid solution depends upon the concentration of acid present and upon the strength of the acid, i.e., the relative tendency of the acid to be reactive. It is possible to have a large total acidity with a slight reaction (concentrated solutions of weak acids) or a strong reaction with a relatively low concentration of acid (dilute solutions of strong acids).

Similar considerations apply to solutions of bases where the reactive substance is a compound group or radicle called hydroxyl. We can then speak of the reaction of a base. Now, it is obvious that although solutions with strong basic reaction are quite different in properties from those of a similarly strong acid reaction, solutions of very weak acidic or basic reaction are less markedly different. It is conceivable that there may be some intermediate situation where there is neither an acid nor an alkaline reaction. Solutions exhibiting this property are said to have a *neutral* reaction or to be neutral. Since there is no essential discontinuity as we proceed from acid reactions through a neutral point to alkaline reactions, it has been found desirable to use a continuous scale of measurement and to speak of a point on this scale as simply the reaction.

Various scales have been devised. A convenient one is the pH scale which is arranged so that very highly acid reactions are assigned small values and very alkaline reactions higher numbers. The neutral point is at pH 7.0, i.e., a solution of pH 7.0 is neutral and exhibits neither an acid nor an alkaline reaction. A solution of pH 6.9 is slightly acid; one of pH 7.1 is slightly alkaline. The approximate pH values of a number of solutions are listed below:



*Approximate pH values*

Dilute solutions of strong acids....	1-2	} Very acid
Vinegar (5 per cent acetic acid)...	2.5	
Saturated boracic acid solutions....	4.5	
Sugar cane crusher juice.....	4.5-5.0	
Distilled water, aerated.....	5.5-6	} Neutral
Distilled water, boiled (pure water)	7.0	
Tap water in Honolulu.....	7.5-8.0	
Dilute solution of baking soda.....	8	} Very alkaline
Dilute solution of borax.....	9	
Lime water .....	10.5	
Dilute solution of washing soda...	12	
Dilute solution of soda lye.....	13-14	

## SOIL REACTION (10, 18)

Soils generally range in pH from 4 to 10, with most agricultural soils falling within the narrower limits of 5 to 9. A soil with a pH of 7.0 is said to be neutral and has neither an acid nor an alkaline reaction. A neutral soil is not, however, saturated with bases to the exclusion of replaceable hydrogen. A soil can be freed from all replaceable bases by various means and is then extremely acid. If a basic substance is then gradually added to the soil the pH will increase, the replaceable hydrogen decrease, and the replaceable bases increase progressively until theoretically there is attained a quite high pH value at which all the replaceable hydrogen has been exchanged for the base. Actually, there is no practical way to reach this point because the soil is decomposed at high pH values. Accordingly, some arbitrary pH, say 7.0, must be chosen at which the process is terminated.

No particular change in the soil characteristics will necessarily take place at or near a pH of 7.0. (For a given soil there is a specific pH value, called the isoelectric point, at which changes in soil properties are quite marked. This value is rather low for most soils.) However, the habit of considering a pH of 7.0 as a unique value (induced by work with strong acids and bases) has become so fixed that most base-exchange studies are pursued with solutions at this pH. It is customary to equate the total acidity, i.e., the amount of acid neutralized to a pH of 7.0 to the replaceable hydrogen. The conventional values for replaceable hydrogen are thus less than the actual replaceable hydrogen.

## BUFFERS (2, 3, 5)

Whenever a base is gradually added to an acid the pH increases progressively, but not necessarily at a steady rate. As an example, consider the neutralization of muriatic acid by ammonia. When the solution has been brought to a pH between 2 and 3, moderately large amounts of ammonia can be added with small changes in pH. The solution is then said to be *buffered*. It is protected against rapid fluctuations in pH. Any material having these properties is called a buffer. Buffers are very useful; they are found in nature and used in the arts and industry. By buffering a solution used in industrial processes, stability is attained. The blood is a

highly buffered medium. If it were not buffered carbonic acid produced in the tissues and acids obtained in foods would exert disastrous effects.

Although a solution of muriatic acid partially neutralized with ammonia is buffered at a pH between 2 and 3, further additions of ammonia will gradually increase the pH. As we approach a pH of 4.4 the conditions become reversed and at this point a very small addition of ammonia will cause a large change in pH. The solution now is said to be only very slightly buffered.

In general, soils are quite highly buffered throughout their normal pH range. Accordingly a soil with a pH of 6 may have a fairly high "lime requirement," i.e., a large application of lime may be required to bring the soil to a pH of 7.0. Soils are thus protected against great changes in reaction and the effect of altered conditions, particularly those due to applications of acid fertilizers, is minimized.

#### HOW HAWAIIAN SOILS HAVE BECOME ACID OR ALKALINE

The original rock materials from which Hawaiian soils have been formed consisted of basaltic lavas and (near the sea coast) coral. Both of these materials were essentially basic in character. Hawaiian basalts are composed largely of silica, iron, aluminum, and various bases, principally sodium, calcium, and magnesium (14, 17). Coral is essentially carbonate of lime.

The mechanical and chemical changes due to rainfall, running water, variations in temperature, etc. constitute a process described as weathering. Weathering has resulted in the disintegration and transformation of the rock into soil. The effect of rain water containing various gases has been to partially dissolve the disintegrated fragments thus released so that at an early period of formation the soil must have been quite alkaline (10).

In regions of low rainfall weathering has been slow and the soil remains in an early stage of development. It may still be alkaline. Thus, soils in the Kau region are still alkaline or neutral (10).

In regions of heavier rainfall the bases were carried below the surface soil because of the downward movement of water (6). They also dissolved a large part of the original silica which was also leached out. The result has been a gradual depletion of the soil with respect to silica and the bases sodium, potassium, calcium, and magnesium with a relative enrichment with regard to iron, aluminum, and replaceable hydrogen. As the bases have been removed the soil has become less alkaline.

Gases dissolved in the rain water, acids formed by microorganisms and by decaying vegetation have gradually made the soils more acidic, thus accelerating the removal of bases. This process has gone on at all times and in all places, but most rapidly in regions of warmth, heavy rainfall, and dense vegetation. At the present time a great part of our middle-~~bel~~ and upland soils are distinctly acid.

Along the sea coast this process has been retarded by the factors of less rainfall and vegetation, but principally by the presence of coral rock fragments. Carbonate of lime (calcium carbonate) is basic, but not very soluble at a pH above 7.0. It is not readily removed by leaching but can still neutralize acids which are formed in the soil over a long period of time (21). Furthermore, coral provides a source for

replaceable calcium. Coral soils thus tend to be alkaline and rich in replaceable calcium.

In the arid regions of the southwestern part of the United States the process of weathering, while slower than in more humid sections, has over many years formed soils which physically may be in a late stage of development. Lack of adequate rainfall has, however, reduced leaching of bases so that at present these soils are saturated with carbonates and sulfates of sodium, calcium, magnesium, etc. These soils are typical alkali soils (7, 10, 20) and are not generally found in Hawaii. We do find, nevertheless, a few soil areas near our sea coasts formed from recently drained salt marshes, etc., which are saturated with saline matter, principally sodium salts. Such soils are alkaline, heavy and sticky. When dry they are hard and tend to crack. Although generally well supplied with plant nutrients, they are not usually very fertile until reclaimed by special treatment.

#### EFFECTS OF CULTIVATION

Drainage, plowing, and irrigation accelerate the process of soil disintegration and in that way tend to increase soil acidity. At the same time cultivation improves the soil in other respects. Furthermore, the changes take place slowly. The historic period of cultivation has been short compared with the period during which rocks have disintegrated into soils.

Fertilizers and soil amendments have various effects upon soil acidity (19). Sulfate of ammonia and ammophos form acids in the soil due to a process of bacterial attack upon these ammonium compounds. Nitrates of soda and lime, raw rock and probably superphosphate oppose this process since they introduce permanent bases. Lime, ground coral and coral sand directly neutralize soil acidity (21). Saline irrigation water frequently provides ample supplies of replaceable bases, including potassium as shown by one of us (11).

#### SOIL ACIDITY AND CANE GROWTH

It is a generally recognized fact that plants will not grow well in either very acid or alkaline soils. It is probably safe to say that the amount of total acidity has little direct effect upon growth. On the other hand, soil reaction may be very important, indirectly as well as directly.

The direct effect of low pH has been studied by Martin (13) who grew cane in water cultures which contained fertilizers and were adjusted to various pH values. He discovered increasing evidence of poorer growth below a pH of 5.0, but particularly below 4.0. At pH values higher than 5.0, iron and phosphates were less available and poor growth resulted.

In the soil these conditions may be aggravated by increased solubility of aluminum in acid soils (4). Aluminum has been shown to be toxic in moderately high concentrations. On the other hand, sugar cane may be relatively tolerant to the effects of high acidity when other growth factors are favorable, while poor aeration, lack of sufficient sunlight, water and plant food, infection with *Pythium*, etc. may augment the effect of acidity (9).

A rather important matter remains to be considered. The bases calcium, magnesium, and potassium are essential to plant growth. A soil with high total acidity is a soil partially depleted of these bases. But of more importance is the fact that a soil of this type does not retain these bases adequately when they are applied as fertilizer because hydrogen is not easily replaced by bases except when they are in an alkaline solution. Acid soils have poor fixing power for potassium, etc., and applied fertilizer bases are readily lost by leaching under conditions of heavy rainfall (12). The problems presented by alkaline soils are of a different character. They are usually well supplied with bases and retain applied potash. Iron and manganese are generally less available and chloroses of one type or other may be caused by deficiencies of these elements in available form.

#### CORRECTIVE AND PREVENTATIVE MEASURES

Excessive alkalinity can be ameliorated by the use of acid-forming fertilizers such as sulfate of ammonia. Commercial yellow sulfur (flowers of sulfur) in the presence of sufficient organic matter will tend to reduce alkalinity (22). Areas of land formed from swamps near the sea can be reclaimed by adequate drainage, including ditches and subsurface drains, and by irrigation. The appropriate type of irrigation water is moderately saline. Sodium-saturated soils are of a heavy type and are only kept open by the presence of some salt in the water. Pure mountain water will render these soils impervious.

It is a matter of common knowledge that excessive acidity can be corrected by applications of lime in one or more of its various forms (21). Lime both neutralizes the acidity and supplies the soil with replaceable calcium. The effect of such treatment is usually beneficial and it is customary in many countries (formerly including Hawaii) to apply large amounts of lime to acid soils.

However, it has been shown by Verret (24) that heavy liming of sugar cane lands probably results in poor juices for the succeeding crop. As a result, liming as an amendment when used in very large quantities on some Hawaiian soils has not been immediately successful and is not practiced so extensively at present as in the past. It is, of course, conceivable that a beneficial effect can be obtained by rather moderate periodic applications of lime. It is probably neither necessary nor desirable to apply sufficient lime to bring the soil to neutrality. A policy which would prevent increase of acidity and maintain a reasonable supply of replaceable (available) calcium appears to be desirable (12). From this point of view, periodic rapid chemical estimations of "available" calcium may be very useful, while determinations of lime requirement would be of little or no advantage.

A tendency for soil acidity to increase can be checked to some extent by the use of fertilizers which are not acid-forming, such as raw rock, bone meal, nitrate of lime, or superphosphate (19). Under certain conditions, organic matter yields bases to the soil upon decomposition. In nature the continuous decomposition of root residues probably has this effect to some extent. However, the influence of organic matter upon soils is very complex, since it depends upon climate, rainfall, the types of microorganisms in the soil, the nature of the organic material and other factors.

An acid soil, low in replaceable bases, in a region of high rainfall does not adequately retain applied potash and ammonia nitrogen. The problem is that of obtaining maximum value from fertilizer by providing it to the crop rather than to the drainage. It has been shown by Ayres (1) and others that at certain periods in the life of the cane there is a maximum tendency to assimilate potash and nitrogen. It is possible that these factors definitely affect the optimum utilization of fertilizers and that questions of time and number of applications are most advantageously considered with due regard to these facts.

At the present time a comprehensive study of soil changes affected by various types of fertilization, particularly with reference to soil acidity, is being pursued by P. L. Gow. It is hoped that an augmented knowledge of the rate of increase or decrease of soil acidity will enable us to predict the outcome of different fertilizer practices over a period of years and to suggest alternative means for controlling undesirable changes.

#### SUMMARY

1. A general discussion of acidity and alkalinity is presented: Strong acids have certain easily recognized characteristics, such as sour taste. Bases are substances which neutralize acids and acids are substances which neutralize bases.
2. The total acidity of a soil is equivalent (a) to the amount of base required to neutralize the soil, and (b) to the conventional value for replaceable hydrogen.
3. Soil reaction is a characteristic due to the amount of acidity or alkalinity in a specially active form. It is evaluated on the pH scale, with strongly acidic reactions assigned low numbers and alkaline reactions higher numbers.
4. A buffer is a substance in a relatively stable state so that large changes in pH are not readily produced by small or moderate changes in total acidity. Soils are usually highly buffered.
5. Hawaiian soils appear to have been originally alkaline. During the process of weathering and transformation they have tended to become acid with a consequent loss of bases and silica and a relative enrichment with respect to aluminum, iron and replaceable hydrogen. Along the sea coast coral rock has checked this process. Reclaimed salt marsh land is alkaline.
6. Certain fertilizers and soil amendments tend to increase soil acidity; others tend to check it.
7. Excessive acidity or alkalinity may be directly and indirectly deleterious to the growth of sugar cane.
8. Excessive alkalinity may be checked by the use of acid-forming fertilizers or soil amendments. Acidity may be controlled by the use of proper fertilizers and reduced by the use of lime.
9. An acid soil is generally deficient in replaceable bases essential to cane growth and furthermore does not adequately retain applied fertilizer bases. This fact may have an important bearing upon the optimum time and number of applications of such fertilizers.

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# The Fluctuations of Sugars in the Leaf Sheaths of the Sugar Cane Plant During the Day and the Night

By CONSTANCE E. HARTT

## CONTENTS

	Page
I. Historical Introduction .....	330
The transport of sugar in plants is of fundamental importance.....	330
Translocation is the movement of materials through plants.....	330
Translocation of sugar occurs in the phloem.....	330
Translocation occurs both day and night.....	331
What external and internal factors affect the speed of translocation of sugar?.....	331
The mechanism of translocation is unknown.....	333
What kinds of sugars are translocated?.....	334
II. Original Research.....	334
Methods .....	334
Results .....	335
Discussion .....	341
Moisture .....	341
Cane sugar .....	341
Simple sugars .....	342
Polysaccharides .....	343
Starch .....	344
What is the translocation form of sugar in the sugar cane plant?.....	344
The sucrose reducing sugar ratios in blades and sheaths.....	347
Sugar gradients .....	349
What factors affect the translocation of sugar in the sugar cane plant?.....	350
Summary .....	353
Acknowledgment .....	353
Literature cited.....	353



## I. HISTORICAL INTRODUCTION

### THE TRANSPORT OF SUGAR IN PLANTS IS OF FUNDAMENTAL IMPORTANCE

Questions concerning the origin of cane sugar in the leaf blades of the sugar cane plant were considered in a previous paper (15) on the fluctuations of sugars in the leaf blades of the sugar cane plant during the day and the night. Of equal importance are the many questions to be raised regarding the origin of cane sugar in the millable cane. In what form does the sugar migrate from the blade to the stem? When does the movement or translocation take place? Through which cells does the sugar pass? By what means does the sugar move? What external and internal factors hasten the translocation of sugar, and in that way promote the storage of sugar in the millable cane? Do any of the constituents of fertilizers or soil amendments have an effect upon the rate or efficiency of translocation? What is the effect of irrigation or drying upon the movement of carbohydrates\* from the blade to the stem?

Because all sugars in moving from the blade, or place of manufacture, to the stalk or place of storage, must pass through the sheath in some form or other, a study of the variations in the carbohydrates in the sheath during the day and the night was undertaken in an attempt to elucidate some of the problems proposed in the preceding paragraph. The results of our study are offered now as a report of progress and are not intended as an exhaustive study upon which applications should be based. Further studies of a similar nature will be recorded as they mature.

For the non-botanical reader a few explanations regarding the process of translocation are presented as an introduction to the report of the original research.

### TRANSLOCATION IS THE MOVEMENT OF MATERIALS THROUGH PLANTS

The scientific term applied to the migration or movement of dissolved substances through the plant is translocation. This process is as important for the well being of a plant as is the circulation of the blood for the human body. The complex body of a plant such as sugar cane is composed of several kinds of organs each with special functions. For the proper carrying out of these functions every living cell must be supplied with water, essential salts, sugar, and other things. The tiny root tips poking their way deep into the soil must receive a constant supply of sugar to enable them to continue growth. Even the processes of absorption of nitrogen, phosphorus, potassium, etc., by the roots are thought to require a plentiful supply of sugar, inasmuch as they are aided by the energy released in respiration (16). Surplus sugar over and above that used in growth is stored in the stem. As an eye sprouts and forms a young shoot on a cutting, translocation of sugar from the cutting to the growing points of both stem and root is essential. Thus the process of translocation of sugar is of fundamental importance.

### TRANSLOCATION OF SUGAR OCCURS IN THE PHLOEM

The question of which cells are responsible for the downward movement of sugar from the leaves to the stems has been studied recently by Mason and Maskell (24),

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\*For brief definitions of the carbohydrates mentioned in this report, consult the previous paper (15).

who investigated the cotton plant. This is a particularly favorable kind of plant for such studies because of its internal structure. The two tissues which, because of the elongation of their cells, might theoretically form the pathway for translocation (phloem and xylem) are separated in the cotton plant by a thin-walled tissue which breaks easily (the cambium); thus a separation of the phloem and xylem can readily be made before analysis. This has been done by Mason and Maskell, who found that sugars fluctuate in the phloem during the day and the night in the same way that they do in the leaves, but that they remain relatively constant in concentration in the xylem. They conclude that the translocation of sugars takes place in the phloem. Curtis (6) also is of this opinion.

The structure of the sugar cane plant differs from that of cotton. In sugar cane, the phloem and xylem occur close together with no separating tissue, and little bundles or groups of phloem and xylem cells are found in the veins and midrib of the blade, in the fibers of the sheath, and scattered throughout the stem. Because of the impossibility of separating the phloem and xylem in sugar cane for the purpose of quantitative analysis, and because the methods for microchemical study are not entirely satisfactory, the determination of the pathway of translocation can not be made well with sugar cane. However, inasmuch as the studies of Curtis and of Mason and Maskell and of others furnish strong evidence that the phloem is the pathway of translocation in other kinds of plants, this seems a safe assumption in sugar cane.

#### TRANSLOCATION OCCURS BOTH DAY AND NIGHT

We are accustomed to think that sugar is made and stored in the blade during the hours of daylight and translocated to the stem during the night. The question arises as to whether some translocation of sugar occurs also during the day. This problem has been studied by Tschesnokov and Bazyrina (33), who found that some kinds of plants carry on translocation chiefly during the night (e. g., the potato), others (e. g., peas) principally during the day. Colin (2) in a study with the sugar beet, found that sugar migrates from the blade to the root by day as well as by night. The question of time of translocation in sugar cane is considered in this report.

#### WHAT EXTERNAL AND INTERNAL FACTORS AFFECT THE SPEED OF TRANSLOCATION OF SUGAR?

Because of the uncertainty regarding the kind of sugar which is translocated and for other reasons, the factors affecting translocation are not thoroughly understood. Some of the more important factors are the relative amounts of translocatory material, the necessity for living cells in the phloem, length of day, and water content. Many studies with several kinds of plants have shown that in passing from the leaves to the stems, sugars go from a place of low concentration to a place of high concentration, thus against the concentration gradient, or "upstream." A recent illustration of this phenomenon is afforded by the results of Leonard (21), who found that in the sugar beet, cane sugar usually increased continuously from the blades to the roots. The data presented in this paper seem to indicate the same fact, and it is well known that there is more cane sugar in the stem than in the leaf of sugar cane. If cane sugar is a translocatory substance, how can it move against the gradient? Some help in understanding this problem may be obtained from the work of Mason and Maskell (24), who found that the separate analysis of tissues enabled the detection of concentration gradients in the cotton plant, with the sugar

moving with the gradient, or "downstream" in the phloem of the stem. They found that even when sugar moves against the gradient an increased concentration of sugar in the leaf caused an increased rate of translocation to the stem. The same investigators (26) suggest that where the food materials appear to move "upstream," perhaps the "downstream" movement of mobile material is masked by a large amount of storage or immobile material. However, there is at least one place in the cotton plant in which sucrose definitely accumulates against a gradient, and that is in passing from the cells of the mesophyll (or middle, green tissue of the leaf) into the phloem of the fine veins in the leaf, according to Phillis and Mason (29), who have suggested that the transition cells of the fine veins and the companion cells of the phloem are responsible for the accumulation of sucrose against a gradient and probably also for the movement of glucose and fructose against a gradient. They state that their experiments demonstrate that although concentration gradients may determine the direction of movement along the larger veins, yet there are other factors that determine the direction of movement between the mesophyll and the fine veins.

It was recognized very early that living cells are essential for translocation and a review of the evidence indicating that assumption is given by Curtis (6). Thus any condition which coagulates the protoplasm or kills the cells of the phloem will interfere with translocation. It has been shown experimentally that local chilling of the petioles (or leaf stalks) to 0-6° C. interferes with translocation; inasmuch as that temperature is never reached in sugar cane countries the effect of low temperatures in decreasing translocation in the sugar cane plant may be considered insignificant. The application of chloroform has also been found to decrease translocation. Of more importance to sugar cane agriculture is the fact that certain nutritional deficiencies, including boron (17, 18) and potassium (9, 10) have been found to interfere with translocation due to the killing of the protoplasm. Sugar cane undergoing certain nutritional disturbances or growing on acid upland soils may have nodal accumulations of iron and aluminum which occur primarily in the xylem, but which may also have a deleterious effect upon translocation in the phloem, although this point is not definitely established.

Insects have been found to interfere with translocation when they injured the phloem; a specific example is the injury due to leaf miners studied by Schneider-Orelli (31). In sugar cane the insect, *Aphis maidis*, which transmits the virus causing mosaic, punctures the cells of the phloem and obtains food in that way. Thus the amount of food to reach the stem may be decreased. There seems to be no evidence that either the virus or the insect causes an actual plugging of the phloem cells. Such a plugging of the phloem accompanied by discoloration occurs in Sereh, a serious disease of sugar cane absent from Hawaii, which in Java was said to interfere considerably with translocation and to cause depressed growth of certain varieties. Leaf scald disease, which occurs in Hawaii, primarily causes a plugging of the xylem and also occasionally affects the phloem; the causal agent of this disease is *Bacterium albidineans*. Whether or not any insects other than the aphids seek primarily the phloem of sugar cane has not been determined.

The effect of water upon translocation as well as photosynthesis in sugar cane is being studied by the writer and a preliminary report has already been published (12). The sheaths of sugar cane plants deprived of water contained more cane sugar than simple sugar, whereas the sheaths of plants adequately supplied with water con-

tained more simple sugar than cane sugar. It may be that the relative amounts of the sugars in the sheath are affected by the amount of water and thus the translocation form of sugar may be dependent largely upon the moisture content.

Another factor affecting translocation is aeration. Puriewitsch (30) and Grünfeld (8) found that the movement of food to and from the cotyledons or seed leaves is dependent upon aeration. Mason and Phillis (27) state that oxygen starvation seems to affect carbohydrate and nitrogen transport to the same extent.

#### THE MECHANISM OF TRANSLOCATION IS UNKNOWN

The chief explanations of the mechanism of the translocation of foods which have been suggested by plant physiologists include diffusion, protoplasmic streaming, mass movement, adsorption, and surface tension.

It has been recognized for some time that diffusion alone is an insufficient explanation because the process is too slow to account for the movement of the amount of sugar known to be transferred. In their studies with the cotton plant, Mason and Maskell (25) found that the rate of diffusion of sugar in the sieve tube is about 40,000 times as great as that for sugar in a 2 per cent solution of sucrose in water and is almost identical with the rate of diffusion of molecules the size of sucrose in air. Although diffusion is not the only mechanism, some method similar to diffusion in that it is affected by concentration, is probable, according to Mason and Maskell (24). These authors suggest that there may be a mechanism in the sieve tube which overcomes resistance to diffusion. In a later contribution, Mason and Phillis (27) state that some theory of activated diffusion is needed. They say that the curtailment of oxygen from a limited region of the bark may completely stop the transport of food, which is resumed when the supply of oxygen is renewed. Therefore they suggest that the energy liberated by respiration may activate diffusion by reducing the resistance of the solvent so that diffusion proceeds in the sieve tube at rates comparable with those in a gas.

That the translocation of foods is due to a mass flow caused by differences in pressure has been proposed by Münch (28) and Crafts (3, 4, 5), both of whose systems have been criticised by Curtis (6) and by Mason and Phillis (27). Crafts has suggested that the mass flow of food takes place through the walls of the sieve tubes as well as through the interiors of the cells.

The contents of some cells when viewed through the microscope are seen to be in rapid motion. The protoplasm and chloroplasts (small bodies containing the green pigment chlorophyll) circulate around the cell. This movement is not observed in all cells, possibly because of damage during the preparation of the materials for observation or on account of age or diseased condition of the cell. This motion, called protoplasmic streaming, is sometimes observed in the phloem. If such a movement were universal in active phloem it might carry soluble food from one end of a cell to another far more quickly than can be done by diffusion. The food could then pass through the pores in the end walls (unless these pores are plugged) and be swept rapidly by the streaming protoplasm to the opposite end of the cell. However, the theory that the means of translocation is protoplasmic streaming is not entirely satisfactory, since it does not account for the cause of protoplasmic streaming. Van den Honert (34) has even suggested that the streaming of protoplasm may be the result of translocation rather than its cause. Kok (19) studied the rate of

translocation and concluded that there is no evidence that protoplasmic streaming aids translocation.

One of the simplest theories of translocation is the one recently proposed by Van den Honert (34), depending upon the fact that things which reduce surface tension between two substances move to the boundary. The speed of such a movement is about of the same order of magnitude as the speed of translocation. This theory is satisfactory not only from the standpoint of speed but also because it is in harmony with the dependence of translocation upon differences in concentration.

Went (36) has suggested that the "growth promoting substance" which is a newly discovered plant hormone essential for growth, may be translocated through the companion cells of the phloem by catephoresis. Catephoresis is the movement of an electrolyte (salt, for example) through a solution through which an electric current is passed, in the direction of one of the poles. Catephoresis results in the accumulation of the electrolyte in one place, and there is a movement from a solution of weak concentration to a highly concentrated location. Electric currents have been found in plants by various investigators and the suggestion has been made that electrical phenomena may aid in the process of translocation. Phillis and Mason (29) remarked that since a non-electrolyte like sugar can be accumulated against a gradient by the fine veins perhaps electrical forces are not at work. The possible effect of electric currents upon translocation has recently been mentioned by Lund (23), who has demonstrated electric circuits between different microscopic points on the same cell. Differences in electric current between larger areas in the stems of sugar cane may be measured by the electrynx, by which the Brix may be estimated, giving a measure of the ripeness of cane. The electrynx is now in use on a few plantations in Hawaii.

According to Crafts (5) a combination of diffusion, protoplasmic streaming, and pressure flow seems to explain translocation in the potato most satisfactorily.

Whether these theories of the mechanism of translocation will be permanently accepted in the literature remains to be seen.

#### WHAT KINDS OF SUGARS ARE TRANSLOCATED?

It is known that foods to be translocated must be in solution. Since starch is a solid, it cannot be moved without first being digested to sugar. The question arises as to what is the sugar of transport. Are the simple sugars translocated, or sucrose, or both?

Studies of the translocation forms of sugars have been conducted by many workers with different kinds of plants. Some investigators have presented evidence that the simple sugars are transported, others that cane sugar is transported, and some have favored the view that both the simple sugars and cane sugar may be transported. Lists of the authors favoring each view need not be included here. This subject has been discussed recently by Leonard (20).

With sugar cane, Viswanath (35) in 1919, favored the view that the simple sugars are translocated, whereas Geerligs (7) in 1924 concluded that both cane sugar and the simple sugars are translocated in the sugar cane plant.

## II. ORIGINAL RESEARCH

### METHODS

Details regarding the plants used in this investigation, the methods of sampling and of analysis, have already been recorded (15). The leaf of the sugar cane plant

consists of two distinct portions, the blade and the sheath. The blade is the flat, dark green part which is extended in the air and in which the greater part of the manufacture of food occurs. The sheath is the part which is clasped tightly around the stem. The transport of water and fertilizer elements from the stem to the blade, and the movement of sugars and several other foods from the blade to the stem, are two important functions of the sheath. The sheaths of the same leaves discussed in the former paper were used in this investigation, which really constitutes a continuation of the study of photosynthesis. No analyses of sheaths were made in the June experiment. In the December experiment analyses were made of the midribs removed from the leaves taken at 7 a. m. and at 7 p. m.

## RESULTS

The results of the analyses of simple sugars, cane sugar, polysaccharides, and moisture of the sheaths taken in April 1933, are recorded in Table I and Fig. 1. The curves have been smoothed by the method of moving averages, and it is felt that comparisons made with graphs smoothed in this way are more reliable than the original data because this method tends to overcome the effects of individual differences and sampling errors. The results of the analyses of the sheaths taken in the December 1933 experiment are given in Table II and Figs. 2-4. The results of the analyses of the midribs taken in December are presented in Table III. Sunlight records measured by an Eppley pyrheliometer were published in the other paper (15). The results of the December experiment are expressed upon the residual dry-weight basis. The residual dry weight, which is calculated by subtracting the sum of the total sugars plus polysaccharides from the dry weight, remains relatively constant over short periods of time (24-48 hours), and therefore constitutes a more reliable basis for calculations than either the green-weight or the dry-weight basis.

TABLE I

Analyses of sheaths taken in April 1933. Sugars and polysaccharides expressed on moisture-free basis. Also moisture percentages.

Time of sampling	Simple sugars	Cane sugar	Polysaccharides	Moisture
	Per Cent	Per Cent	Per Cent	Per Cent
April 25				
1 p.m. ....	$3.78 \pm 0.002$	$7.80 \pm 0.248$	$23.17 \pm 0.443$	$79.78 \pm 0.066$
3        .....	$3.29 \pm 0.023$	$7.83 \pm 0.019$	$20.94 \pm 0.057$	$79.19 \pm 0.009$
5        .....	$3.56 \pm 0.095$	$8.99 \pm 0.042$	$21.67 \pm 0.128$	$78.79 \pm 0.076$
7        .....	$3.33 \pm 0.009$	$9.07 \pm 0.119$	—	$80.34 \pm 0.076$
9        .....	$3.82 \pm 0.002$	$8.37 \pm 0.009$	$21.00 \pm 0.195$	$80.72 \pm 0.090$
11       .....	$3.56$	$9.82$	$24.78 \pm 0.114$	$80.57 \pm 0.171$
April 26				
1 a.m. ....	$3.06 \pm 0.057$	$8.57 \pm 0.114$	$23.60 \pm 0.057$	$80.38 \pm 0.142$
3        .....	$3.23 \pm 0.009$	$7.98 \pm 0.019$	$23.33 \pm 0.028$	$80.07 \pm 0.195$
5        .....	$3.96 \pm 0.014$	$10.35 \pm 0.119$	$21.38 \pm 0.333$	$81.97 \pm 0.682$
6        .....	$3.59 \pm 0.023$	$9.01 \pm 0.195$	$20.97 \pm 0.162$	$80.36 \pm 0.104$
7        .....	$3.55 \pm 0.019$	$8.25 \pm 0.295$	$20.94 \pm 0.033$	$79.88 \pm 0.066$
9        .....	$4.36$	$8.00$	$21.01$	$80.10 \pm 0.081$
11       .....	$4.01 \pm 0.023$	$9.22 \pm 0.081$	$24.56 \pm 0.205$	$80.92 \pm 0.071$

TABLE II

Analyses of sheaths taken in December 1933. Sugars, starch, and polysaccharides expressed on the residual dry-weight basis. Also moisture percentages.

Time of sampling	Simple sugars	Cane sugar	Starch	Polysaccharides	Moisture
December 27	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
5 a.m. ....	9.235±0.135	4.584±0.286	2.329±0.081	31.12±0.622	85.84±0.023
6        .....	10.505±0.131	2.866±0.216	1.723±0.008	26.22±0.257	85.67±0.009
7        .....	11.577±0.074	7.436±0.210	2.075±0.023	27.99±0.350	86.35±0.028
8        .....	9.255±0.051	6.323±0.471	2.197±0.042	29.78±0.340	85.56
9        .....	9.235±0.216	5.271±0.011	2.298±0.086	28.93±1.207	85.71±0.429
11       .....	9.929±0.106	5.441±0.453	3.587±0.207	42.40±1.358	85.00±0.124
1 p.m. ....	10.595±0.020	7.376±0.268	2.670±0.032	40.03±0.033	85.40±0.124
3        .....	9.903±0.150	7.827±0.279	3.414±0.155	36.85±0.125	85.57
5        .....	8.397±0.102	7.044±0.048	3.017±0.050	39.06±0.100	84.09±0.033
6        .....	9.700±0.199	7.993±0.162	2.572±0.045	40.14±1.321	85.58±0.038
7        .....	9.138±0.125	7.614±0.154	2.897±0.003	37.87±0.904	85.01±0.090
9        .....	9.719±0.056	6.935±0.054	2.663±0.024	40.45±0.381	84.93
11       .....	—	—	3.487	38.69±0.319	85.12±0.038
December 28					
1 a.m. ....	8.403±0.051	5.851±0.297	2.528±0.079	39.22±0.079	85.37±0.057
3        .....	7.611±0.044	4.353±0.035	2.460±0.105	30.85±0.074	85.18±0.038
5        .....	9.720±0.122	5.421±0.169	2.425±0.015	38.13±0.098	85.33±0.071
6        .....	10.740±0.340	5.257±0.280	2.789±0.006	34.25±0.223	85.54±0.019
7        .....	9.017±0.106	5.424±0.093	2.224±0.027	28.79±0.904	85.39±0.066
8        .....	7.261±0.144	5.282±0.129	2.023±0.040	29.19±1.307	84.77±0.081
9        .....	7.694±0.079	4.372±0.149	1.957±0.023	31.89±0.212	84.99±0.085
11       .....	8.030±0.112	5.128±0.089	2.015±0.023	28.29±0.508	83.81±0.019

TABLE III

Analyses of midribs taken in December 1933. Sugars, starch, and polysaccharides expressed on residual dry-weight basis. Also moisture percentages.

Time of sampling	Simple sugars	Cane sugar	Starch	Polysaccharides	Moisture
December 27	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
7 a.m. ....	2.617±0.006	2.711±0.179	0.568	24.83±0.438	77.76±0.023
7 p.m. ....	3.503	6.274	0.468	22.98	77.18±0.081
December 28					
7 a.m. ....	3.580±0.042	2.975±0.382	0.594	24.06±0.400	77.68±0.181

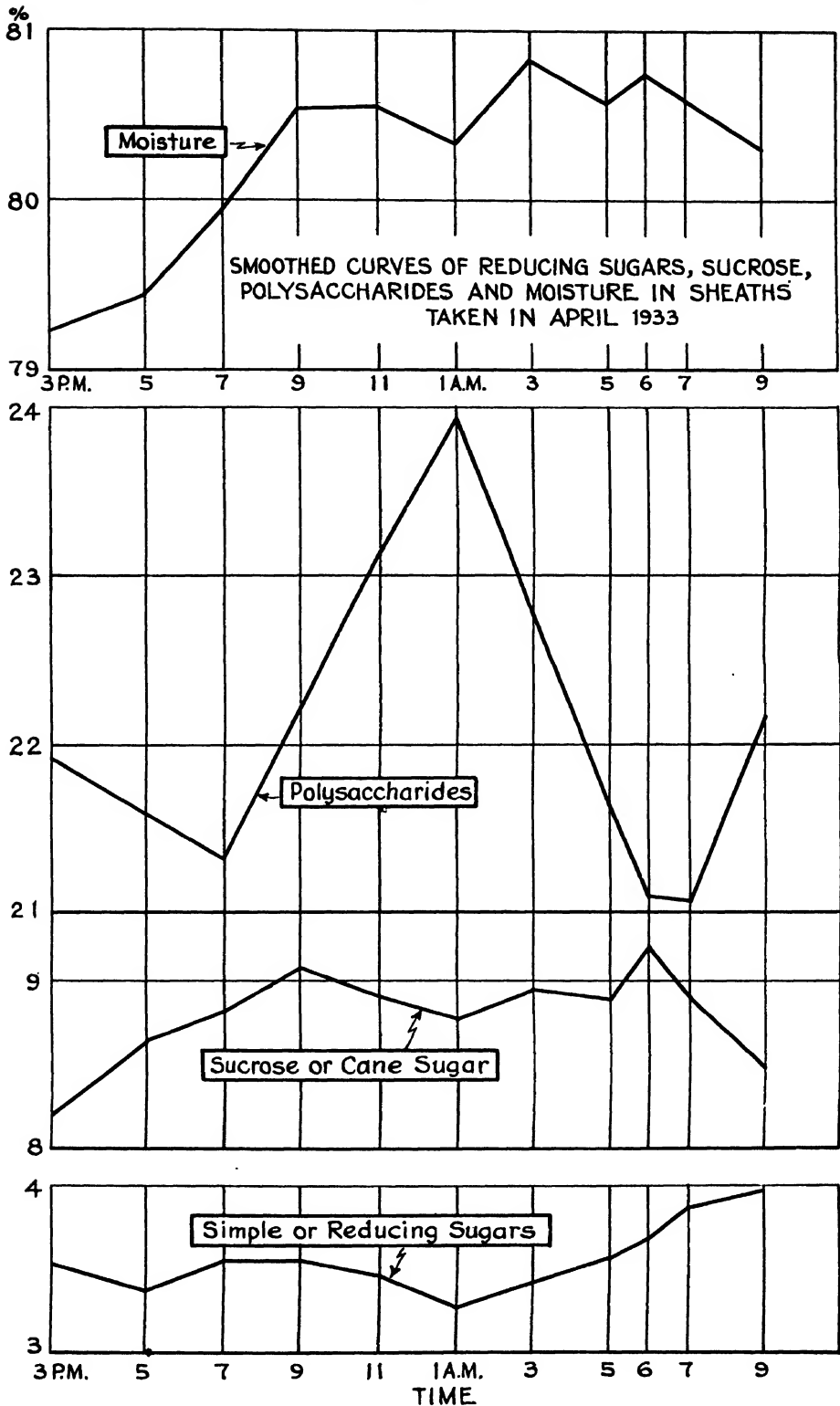


Fig. 1.



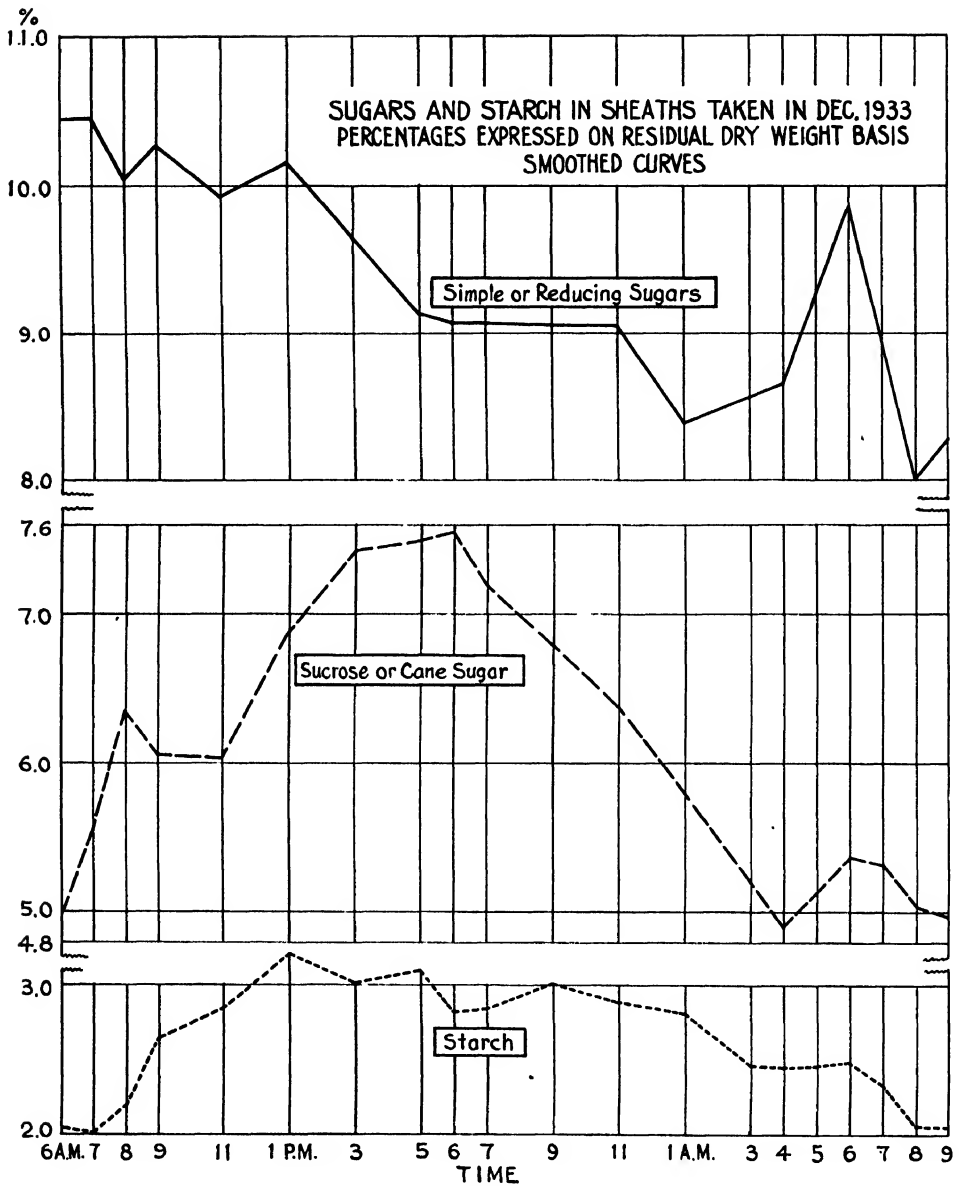


Fig. 2.

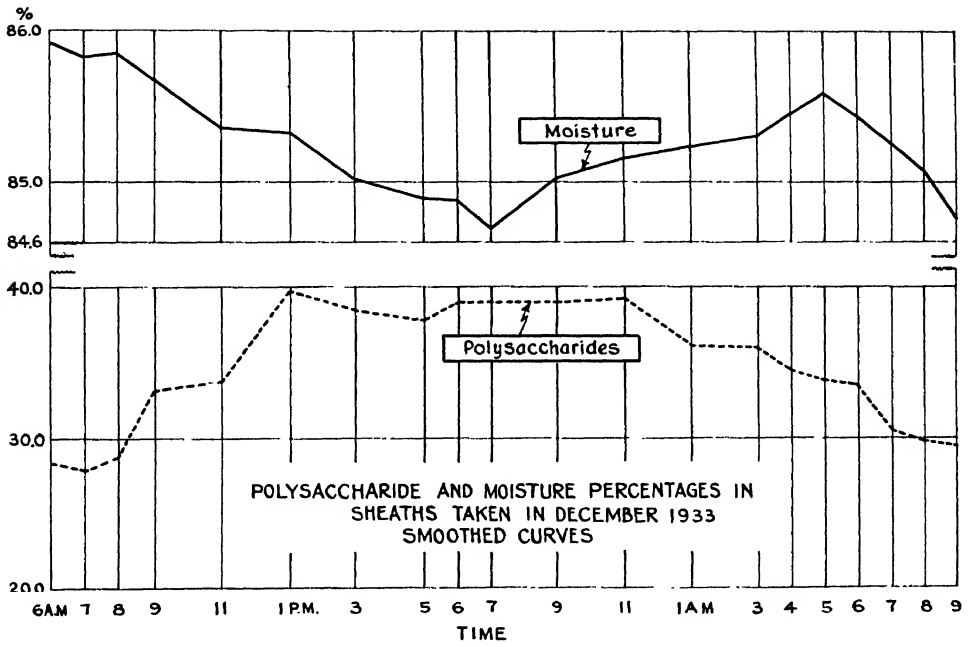


Fig. 3.

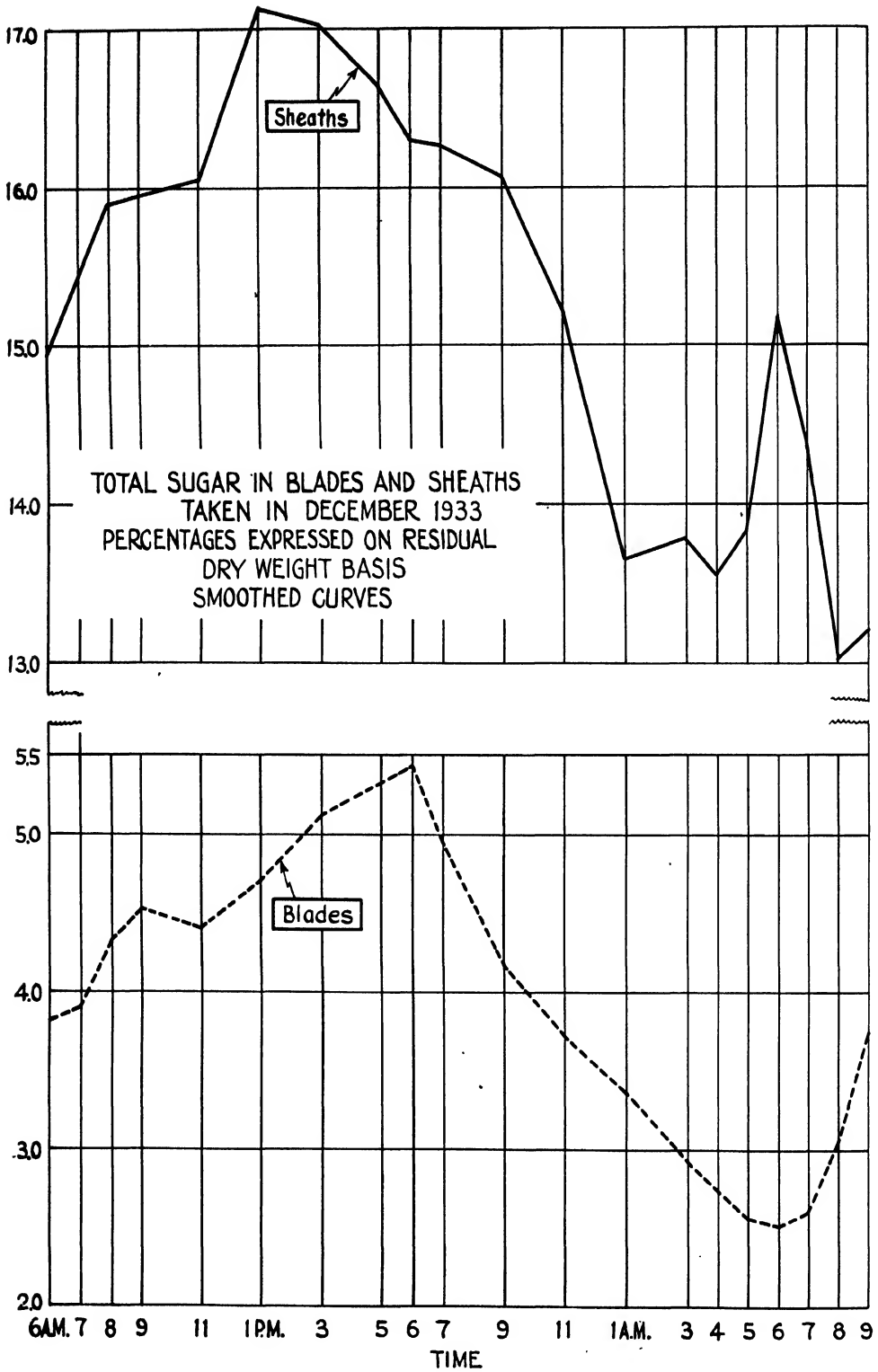


Fig. 4.

## DISCUSSION

*Moisture:*

The water content of the sheaths of the sugar cane plant fluctuates during the day and the night, as shown in Figs. 1 and 3. In the April experiment the moisture percentage rose until 9 p. m. and then remained nearly constant, showing minor fluctuations during the night, and suggesting a decrease in the morning. In the December experiment the moisture content decreased steadily from 6 a. m. until 7 p. m., and then increased until 5 a. m., followed by a decrease until 9 a. m., which was the end of the experiment. Thus the course of the curves for moisture content of the sheaths differed in the two experiments, and both differed from the curves for moisture in the blades (15). They are alike in that both experiments show higher percentages of moisture in the sheaths than in the blades, which may be a point of importance in translocation.

The percentage of moisture in the sheaths exhibited less variation than that in the blades. In the April experiment, the moisture percentage of the sheaths varied from 79 to 81 per cent in round numbers, whereas in the blades the percentage of water varied from about 67 to 70 per cent. In the December experiment, the moisture percentages of the sheaths varied from about 85 to 86 per cent, whereas in the blades the variation lay between 70 and 74 per cent. It would seem that the moisture content of the sheaths is affected by the time of the day less than is the moisture content of the blades. This is only to be expected, since the sheaths are considerably more protected than the blades.

Why did the percentage of moisture in the sheaths remain nearly constant during the night in the April experiment, whereas in the December experiment an increase occurred until 5 a. m.? The moisture percentages of the blades in both experiments showed low points at 3 p. m., high points at 5 a. m., and breaks in the rise during the night. With these similarities in the blades, why did the sheaths differ? The two experiments differed in the age of the plants and in the season. Differences in season (i. e., in temperature and clouds, which affect the rate of loss of water from leaves) were probably responsible for the greater moisture content of the plants taken in December than those taken in April. The plants used in the December experiment were only six months old, while those in April were 10 months of age. Perhaps the sheaths of the younger plants were not as highly cutinized (covered by a thin wax-like coating) as those of the older plants, and thus were more sensitive; or else there may have been some unknown factor responsible for this difference.

*Cane sugar:*

The percentage of sucrose or cane sugar in the sheaths taken in April increased until 9 p. m., remained fairly constant until 6 a. m., and then decreased at least until 9 a. m., as shown in Fig. 1. In the December experiment the cane sugar increased, with some fluctuation, until 6 p. m., decreased until 4 a. m., increased slightly until 6 a. m., and then decreased to 9 a. m., according to Fig. 2. Thus in April the curve for cane sugar in the sheaths resembled that for moisture; but in December the curve for cane sugar in the sheaths resembled that for cane sugar in the blades. Undoubtedly it would be of great interest to understand the full significance of these differences, but at present we are unable to do so.

The content of cane sugar in the sheaths taken in April varied from 8 to 9 per cent on the dry-weight basis; that in the sheaths of the December experiment varied from 5 to 7.6 per cent on the residual dry-weight basis (which gives larger figures than the dry-weight basis). Why was there more cane sugar in the sheaths studied in April than in those taken in December? The answer probably involves both age and moisture content, as well as the fact that more sucrose could be supplied by the blades in the April than in the December experiment. Older leaves generally contain more cane sugar than younger leaves. The sheaths taken in December had higher percentages of moisture than those taken in April; inasmuch as the former also had higher percentages of simple sugars than the latter, it would seem that there was greater digestion of cane sugar to simple sugars in the sheaths containing the greater moisture content since the process of digestion is a union with water. This seems a satisfactory explanation of the lower sucrose content in the sheaths in the December experiment than in the April experiment. A necessary corollary is that at least part of the cane sugar in the sheaths studied in December was removed by digestion rather than by translocation.

The question may arise as to whether any of the fluctuations in cane sugar or simple sugars in the sheaths may be due to photosynthesis in the sheaths. It will be remembered that the green pigment chlorophyll is absolutely essential for the manufacture of food by plants. The sheaths used in these experiments were covered throughout the greater part of their length by other sheaths and therefore were for the most part white or yellowish in color. The small amount of chlorophyll in the exposed portions of the sheaths was insignificant, as shown by the fact that the alcoholic solutions prepared from the sheaths were yellow in color, while those prepared from the blades were a rich, deep green with strong fluorescence (reddish glow). Moreover, it has been shown recently (22) that the amount of photosynthesis conducted by the sheaths of grasses is negligible (and sugar cane is of course a grass). Therefore the fluctuations in sugars in the sheaths were due to factors other than photosynthesis in the sheaths.

#### *Simple Sugars:*

The curves for simple sugars in the sheaths differed considerably in the two experiments. This should probably be expected, since the content of simple sugars in the sheaths is dependent upon many factors. For this reason the two curves will be considered separately.

In the April experiment the simple sugars showed low points at 5 p.m., and at 1 a.m., according to Fig. 1. After 1 a.m., there was a uniform rise until 9 a.m. In some respects the curve seems related to the curve for polysaccharides. At 1 a.m., the simple sugars were at a minimum while polysaccharides were at a maximum, which may indicate a condensation of simple sugars to polysaccharides. Then after 1 a.m., there was a simultaneous decrease in polysaccharides and increase in simple sugars, which may have been due to the digestion of polysaccharides. Such a conversion of simple sugars to polysaccharides would of course remove them from solution which might be an aid in the translocation of additional sugars from the blades.

In the December experiment a very different picture is presented. The greatest percentage of simple sugars occurred at 7 a.m., as shown in Fig. 2; this was followed

by a decrease with some irregularity until 3 a.m.; an increase to another maximum at 6 a.m., and a decrease to 8 a.m. Evidently some cause led to the nearly constant removal of simple sugars from the sheaths until about 3 a.m., when another stronger factor set in resulting in the accumulation of simple sugars in the sheaths until 6 a.m.

In both experiments there was a greater concentration of simple sugars in the sheaths than in the blades, a subject to be discussed more fully below.

As mentioned under *Cane sugar*, there was more cane sugar than simple sugar in the sheaths taken in April, but in the December experiment there was more simple sugar than cane sugar. This may have been due largely to the greater percentage of moisture in the sheaths taken in December, leading to greater hydrolysis or digestion of sucrose than in the April experiment.

### *Polysaccharides:*

The most striking feature of the curve for polysaccharides in the April experiment is the maximum at 1 a.m., shown in Fig. 1. Because it occurs at the time of minimum concentration of simple sugars in the sheaths and minimum concentration of polysaccharides in the blades, one is led to the conclusion that the polysaccharides are removed from the blades during the night by hydrolysis and subsequent translocation of simple sugars, some of which upon reaching the sheaths are converted into polysaccharides again. Such a temporary storage of polysaccharides in the sheaths during the night might be of considerable importance in removing carbohydrates temporarily from solution thus permitting the continued translocation of soluble carbohydrates from the blades into the sheaths.

In the December experiment, polysaccharides in the sheaths rose to a maximum at 1 p.m., remained high until 11 p.m., and then decreased until 9 a.m., according to Fig. 3. Here again there seems to be a relationship between the period of high concentration of polysaccharides (1-11 p.m.) and a period of decreasing concentration of simple sugars (1 p.m.-3 a.m.), which may indicate a conversion of simple sugars to polysaccharides for temporary storage during the afternoon and night. Some factor caused a rapid increase in simple sugars beginning about 3 a.m. The cause may have been a complex of factors, including the more rapid increase in water content beginning at 3 a.m., and also the decrease in polysaccharides in the sheaths which began after 11 p.m. It is suggested that due to the high but decreasing content of simple sugars in the sheaths in the early morning, a condensation of simple sugars occurred resulting in the formation of polysaccharides. This condensation by removing soluble sugars from solution permitted further translocation from the blades to the sheaths. Simple sugars may also have been continuously leaving the sheaths. Loss of simple sugars by translocation out of the sheaths and by condensation resulted in a minimum concentration of simple sugars. When, however, the amount of simple sugars coming in from the blades plus the amount resulting from the hydrolysis of polysaccharides, surpassed the amount removed from the sheaths by translocation, an increase in their concentration in the sheaths occurred.

A comparison of the curve for polysaccharides in the sheaths with that in the blades (15) shows that the concentration of polysaccharides remained high in the sheaths longer than it did in the blades. This is only natural on the assumption

that a temporary storage of polysaccharides in the sheaths aids in their removal from the blades.

Thus the study of the fluctuations of polysaccharides in the sheaths during the day and the night indicates that the simple sugars are translocated and that a temporary storage of polysaccharides in the sheaths during the night aids in the transportation of carbohydrates from the blades to the stems.

#### *Starch:*

Starch was determined in the December experiment only. As shown in Fig. 2, starch increased from 7 a.m. until 1 p.m., and then decreased irregularly until 9 a.m. the following morning. Starch was present in much smaller amounts than the polysaccharides as a whole, indicating the presence in sugar cane of polysaccharides of much greater importance than starch. The curve for starch resembles the curve for polysaccharides as a whole, but is not identical with it.

#### *What is the Translocation Form of Sugar in the Sugar Cane Plant?*

The question at issue is as follows: Is sucrose transported from the blade to the stem, or are the simple sugars translocated, or are both cane sugar and the simple sugars translocated in the sugar cane plant? To study these problems it is necessary to consult the previous paper dealing with the blades (15) as well as the data presented in this report.

We now aim to show from a consideration of the data presented in the two reports that both cane sugar and the simple sugars are translocated in the sugar cane plant.

First let us consider the smoothed curves for total sugars in the blades and the sheaths of the December experiment, as shown in Fig. 4. In the sheaths, the total sugars (cane sugar+simple sugars, estimated as invert sugar) increased in amount from 6 a.m. to 1 p.m., decreased to 1 a.m., remained nearly constant in value until 5 a.m., increased to a second maximum at 6 a.m., decreased to a minimum at 8 a.m., and then increased. These trends will now be considered step by step.

What caused the increase in total sugars in the sheaths from 6 a.m. to 1 p.m.? The possible causes of this increase include the following: (1) photosynthesis in the sheaths, (2) formation of sugar in the sheaths from pre-existing carbohydrates already there, (3) translocation of simple sugars out of the blades, and (4) translocation of cane sugar from the blades. These possibilities will now be considered separately. First, regarding the suggestion of photosynthesis in the sheaths, this may take place only to a slight extent because of the small content of chlorophyll. Second, the suggestion of the formation of sugar in the sheaths from pre-existing carbohydrates already present, is not supported by evidence, because starch and other polysaccharides (the carbohydrates from which sugars might be formed) were both also increasing at the same time with the increase in sugar content. The third suggestion, namely, the translocation of simple sugars from the blades (which we think occurred, as mentioned elsewhere) was probably not the immediate cause of the increase in total sugars, because the simple sugars were decreasing in the sheaths at that time. It is possible that simple sugars coming from the blades were transformed in part into cane sugar in the sheaths; it is, however, unlikely that this

is the sole explanation of the increased sugar content of the sheaths, because as shown in Fig. 5 the relative proportions of the simple and cane sugars remained remarkably uniform in the sheaths throughout the day and the night. There thus remains as the most probable cause of the increased sugar content of the sheaths from 6 a.m. to 1 p.m., the translocation of cane sugar from the blades to the sheaths.

It is also probable that a translocation of simple sugars from the blades to the sheaths occurred from 6 a.m. to 1 p.m. for the following reason. Starch and total polysaccharides in the sheaths increased from 7 a.m. to 1 p.m. Since polysaccharides are not translocatable, their increase must have been caused by a condensation of sugars. Although several workers (1, 32) have presented evidence indicating an interconversion of sucrose and starch, yet it is generally recognized that the chief sugars which condense to form polysaccharides are the simple sugars. Now it is possible that some of the sucrose translocated from the blades was inverted to simple sugars, thus furnishing a supply of simple sugars for condensation to polysaccharides. But the curves show an increase in sucrose and a decrease in simple sugars at that time, thus there is no evidence of a marked inversion of sucrose to simple sugars from 6 a.m. to 1 p.m., the time under consideration. It would seem more probable that the increase in starch and other polysaccharides in the sheaths was due to a condensation of the simple sugars supplied by the blades. Polysaccharides increased from 30 to 40 per cent at that time, whereas simple sugars decreased less than 1 per cent. Because the decrease in simple sugars was not enough to account for the increase in polysaccharides, it seems probable that a translocation of simple sugars from the blades to the sheaths occurred from 6 a.m. to 1 p.m.

Thus it is suggested that both cane sugar and the simple sugars are translocated from the blades to the sheaths during the morning. This is a reasonable expectation, inasmuch as photosynthesis is rapid at that time, insuring a plentiful supply of sugars in the blades, whichever sugar is made first. The increase in simple sugars and sucrose which occurs in the blades during the morning is considered to be the surplus over that which is translocated at the same time.

What caused the decrease in the total sugar content of the sheaths from 1 p.m. to 6 p.m.? It is obvious that this decrease in total sugars is due to the fact that the simple sugars decreased in amount more than cane sugar increased at that time. An examination of some of the life processes of the plant may help in obtaining a physiological explanation of this decrease in total sugars. Two main possibilities occur: (1) perhaps translocation of sugar from the blades ceased after 1 p.m., or (2) utilization and export of sugar from the sheaths may have exceeded the import. The first possibility seems unlikely to the author because cane sugar continued to increase in the blades until 6 p.m., which would seem to indicate a plentiful supply for translocation. Also, sucrose increased in the sheaths until 6 p.m., even though the total sugars decreased after 1 p.m. Thus as long as sucrose accumulated in the blades it also increased in the sheaths. Therefore it would seem reasonable to assume a translocation of sucrose from the blades to the sheaths until 6 p.m., rendering it unlikely that the decrease in total sugars at that time was caused by a cessation in the translocation of sugars from the blades.

The other possible cause of the decrease in the total sugar content of the sheaths from 1 to 6 p.m., namely, that the utilization and export of sugar from the sheaths exceeded the import of sugar into the sheaths, may have been due to three causes:



(a) the formation of starch and other polysaccharides from the sugars, (b) the utilization of the sugar in the sheaths for growth and respiration, or (c) greater translocation into the stems. The cause could not lie in the formation of starch and other polysaccharides from the sugar in the sheaths, because starch decreased at that time and total polysaccharides remained the same or perhaps decreased. Neither is it likely to be due to greater utilization of sugar in growth, because it is generally considered that growth takes place chiefly at night, and because there is no reason for assuming so much greater rate of growth after 1 p.m. than before. While one might assume a greater rate of respiration from 11 a.m. to 3 p.m., because of the increased temperature and the fact that heat increases respiration, yet this does not seem a probable assumption for the period 1 p.m. to 6 p.m., because of the falling temperature. Therefore it does not seem likely that all of the decrease in sugar in the sheaths from 1 to 6 p.m. was due to utilization in the sheaths.

The third possibility, that of greater translocation of sugar from the sheaths to the stems, being the only remaining possibility, would seem to be the chief explanation of the greater export than import of sugar in the sheaths from 1 to 6 p.m. This, however, is of no help to us in our main problem (to find which sugar is translocated) inasmuch as it is equally possible that the simple sugars were translocated as such, or that they were converted into sucrose before translocation. However, since there is evidence that both cane sugar and the simple sugars were translocated from the blades to the sheaths during the morning, it seems possible that they were also both translocated from the sheaths to the stems during the afternoon, although it is impossible to prove it from the data at hand.

What caused the decrease in total sugars in the sheaths from 6 p.m. to 1 a.m.? It is not likely that sugar had ceased entering from the blades, although the amount coming from the blades may have decreased because photosynthesis had stopped. That sugar was passing from the blades to the sheaths after 6 p.m. is strongly indicated by the break in the decrease in sugar in the sheaths which occurred at 6 p.m. coincident with the start of the decrease in the sugar content of the blades. Therefore we may assume that the export of sugar from the sheaths was greater than the import, from 6 p.m. to 1 a.m. Determination of the cause of the greater outgo during this period is not as simple as during the previous period. We know that considerable sugar must be used in growth at this time, because of the general assumption that growth takes place chiefly at night, and because the growing region of a cane leaf is the lower part of the sheath. However, it is unlikely that utilization in place can account for all of the decrease in the sheaths, because we know that considerable sugar eventually reaches the stem, although no analyses of stems were performed in this study. Some slight formation of starch may have occurred, as starch showed a slight but unimportant increase from 6 to 9 p.m. However, the general trend was a decrease in starch, and polysaccharides remained the same from 6 to 11 p.m. and then decreased; therefore it is improbable that the decrease in total sugars was due chiefly to condensation. In short, the suggestion is made that between 6 p.m. and 1 a.m. sugar was used in respiration, growth, and in translocation.

Having shown that a translocation of sugar from sheaths to stems may have occurred from 6 p.m. to 1 a.m., there follows the question as to which sugar was translocated. Now it is interesting that from 6 to 7 p.m., coincident with the first marked decrease in cane sugar in the blades, an increase in simple sugars was found.

This indicates hydrolysis of sucrose in the blades. At that time the simple sugar content of the sheaths discontinued its decrease and remained nearly constant, which is only to be expected if the sheaths were receiving a steady supply of simple sugars from the blades. Thus it would seem that in this experiment sucrose was translocated into the sheaths as long as there was a supply available in the blades, and that during the night the simple sugars were translocated more than sucrose.

From 1 to 5 a.m. the total sugar content of the sheaths remained about the same in amount. This is because the increase in simple sugars was nearly equivalent to the decrease in cane sugar. However, it does not necessarily follow that translocation had ceased, even though the total sugar content remained almost constant. It is possible that both types of sugars were passing on to the stems, and at the same time another process was furnishing a further supply of simple sugars. An idea of this other process may be obtained by an examination of the curves for starch and total polysaccharides, given in Figs. 2 and 3. The sharp decrease in starch from 1 to 3 a.m. probably caused the increase in simple sugars at that time, inasmuch as the hydrolysis of starch leads to the formation of glucose. Considerable hydrolysis of polysaccharides took place after 3 a.m., which would also result in an increase in simple sugars. It seems probable that this hydrolysis of polysaccharides caused the large increase in simple sugars which reached a maximum at 6 a.m., as well as the smaller increase in cane sugar which reached a maximum at the same time. In all probability this early morning increase in sugars represents the surplus over that translocated, inasmuch as there is no good reason for supposing a cessation of translocation at 1 a.m. The results of this investigation neither prove nor disprove the translocation of simple sugars between 1 and 6 a.m. or of cane sugar between 4 and 6 a.m.

After 6 a.m., the total sugar content decreased to a minimum at 8 a.m. This decrease may be explained on the assumption that the greater part of the sugars produced by the hydrolysis of polysaccharides had already been removed by translocation. With the source of supply of sugars diminished by lack of photosynthesis and decreased hydrolysis, export was then greater than import, and the result was a sharp decrease in total sugar content, with both sucrose and the simple sugars lessening in amount. The sugar content of the sheaths increased after 8 a.m., probably because photosynthesis had again furnished a supply of sugars which moved down from the blades.

To sum up, it is suggested that both sucrose and the simple sugars are translocated in sugar cane, that translocation takes place both day and night, and that a temporary storage of polysaccharides in the sheaths aids in the translocation of carbohydrates.

#### *The Sucrose Reducing Sugar Ratios in Blades and Sheaths:*

The ratios between cane sugar and the simple sugars are plotted in Fig. 5 for the April experiment and in Fig. 6 for the December experiment. The December experiment will be considered first because of its longer duration.

In the blades, the ratio was greater than unity at all but two times (4 and 6 a.m.), whereas in the sheaths the ratio was always less than unity. This illustrates in

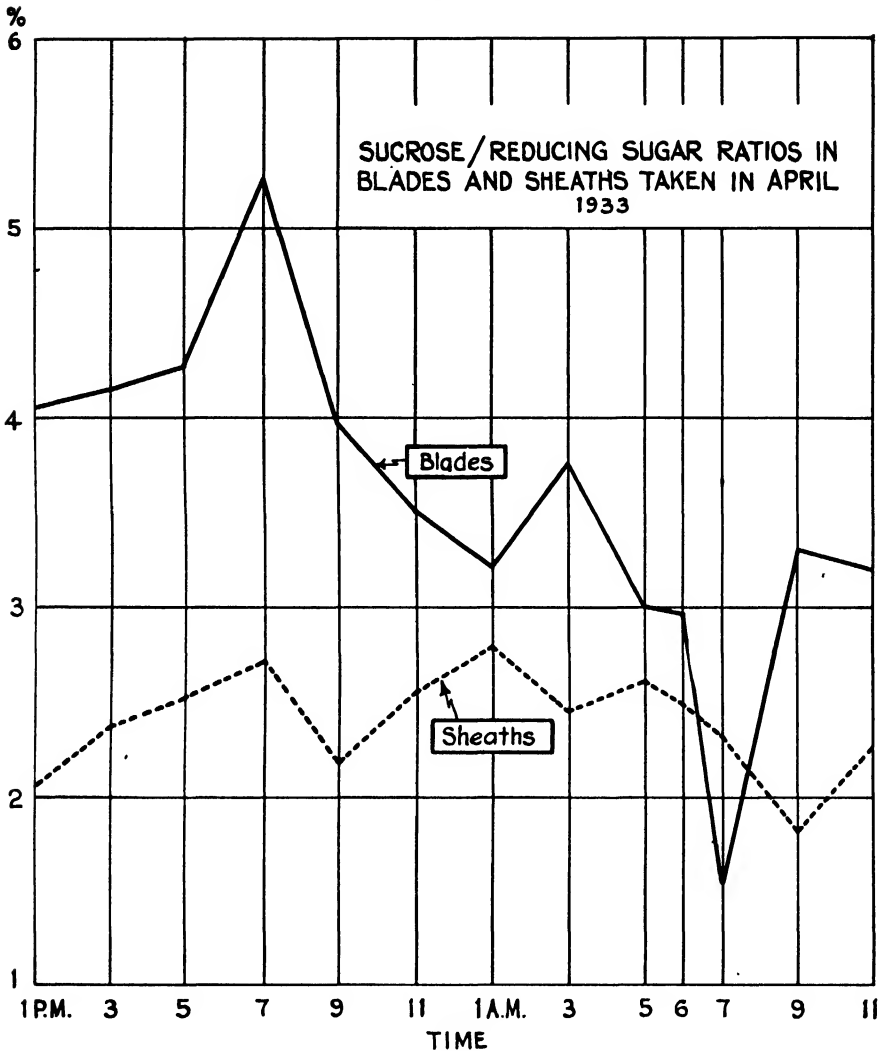


Fig. 5.

striking fashion the predominance of cane sugar over simple sugars in the blades, and the reverse in the sheaths, in the December experiment.

In the blades, the ratio showed no marked tendency up to 3 p.m., fluctuating irregularly but remaining about the same level, indicating no regular change in the relative amounts of the sugars. From 3 to 6 p.m., however, the ratio shows a marked increase, due to the advance in sucrose accompanied by a decline in simple sugars. The curve then falls irregularly from 6 p.m. to 6 a.m., and then rises to 11 a.m. Thus the ratio between cane sugar and the simple sugar in the blades is affected by time of day and probably by light.

In the sheaths, the ratio fluctuates much less than in the blades and is affected little if any by light. The fact that the ratio shows little variation in the sheaths would indicate that there is not much change in the relative amounts of the sugars in the sheaths. In other words, neither formation nor digestion of sucrose is pre-

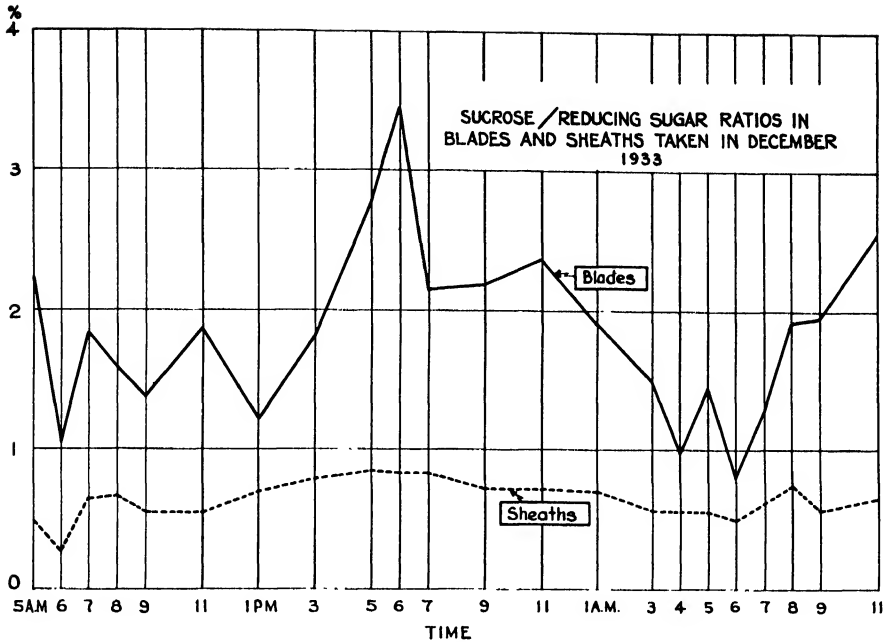


Fig. 6.

dominant in the sheaths; but if some other factor causes a great increase in one kind of sugar, equilibrium is reached again quickly in such a way that there is never any marked fluctuation in the relative amounts of the sugars in the sheaths. This may indicate an important function of the active enzyme invertase often found in the sheaths of sugar cane, to maintain the equilibrium between the sugars.

In the December experiment just discussed there was always more simple sugar than cane sugar in the sheaths, but in the April experiment there was always more sucrose than simple sugars, as shown in Figs. 1 and 5. The April experiment, however, also showed less fluctuation in relation to light in the sheaths than in the blades. Thus whichever kind of sugar is the more abundant, their relative amounts change very little in the sheaths during the day and the night.

#### *Sugar Gradients:*

One of the many interesting questions connected with the translocation of sugar is the effect of the concentration of sugar in the blades upon its translocation through the sheaths into the stems. In passing from the blades to the sheaths to the stems, does sugar move with the diffusion gradient ("downstream") or against the gradient ("upstream")?

The data presented in Tables I and II reveal the fact that both cane sugar and the simple sugars in passing from the blades to the sheaths seem to be passing from a place of lower concentration to a place of higher concentration, when considered on the dry-weight or the residual dry-weight basis.

To study this problem further, the results were recalculated and expressed as grams sugar per 100 grams water. When calculated by this method it was found that in the April experiment both cane sugar and the simple sugars were always

higher in the sheaths than in the blades. In the December experiment, the simple sugars were always greater in the sheaths than in the blades; but cane sugar was greater in the blades than in the sheaths at 12 sampling hours, and less in the blades than in the sheaths at eight sampling hours. Thus even when the concentration of sugar in the water is considered, sugar seems to move "upstream" more often than not.

The results of the December experiment recalculated upon the water basis, for the three hours when the midribs were studied, are presented in Table IV, which shows that the simple sugar content of the midribs was intermediate between that of the blades and the sheaths, with the least sugar in the blades, at these three hours. The cane sugar content of the midribs, on the other hand, was at one time the least, at one time the most, and at one time intermediate. This seems to offer no help in the study of sugar gradients.

TABLE IV

The concentration of sugars in blades, midribs, and sheaths, expressed as grams sugar per 100 grams water.

Time	Blades	Midribs	Sheaths
Simple sugars			
7 a.m.....	.394	.576	1.257
7 p.m.....	.318	.758	1.041
7 a.m.....	.352	.812	1.077
Cane sugar			
7 a.m.....	.734	.603	.656
7 p.m.....	1.018	1.532	.891
7 a.m.....	.439	.629	.643

The problem of sugar gradients has been mentioned in another report (12), "... and the suggestion made that sugars may never actually pass from a cell containing less sugar to one containing more. In both sheaths and stems there is more storage tissue than phloem, and both sheaths and stems have relatively more storage tissue than do blades. The greater percentages of sucrose and reducing sugars in the sheaths and stems than in the blades may be due to the fact that the sugar in the sheaths and stems is spread out in more cells, in which case the direction of translocation would be with the diffusion gradient."

#### *What Factors Affect the Translocation of Sugar in the Sugar Cane Plant?*

Only a beginning can be made in the treatment of this problem, which has not yet been studied adequately. Among the factors which affect the process of translocation in sugar cane the following may be mentioned; insects and diseases, potassium deficiency, the condition of the phloem, the activity of invertase and other enzymes, the supply of sugar, the temporary storage of polysaccharides, and the moisture percentage. The first three of these factors were discussed in the introduction.

The importance of enzymes in facilitating the translocation of foods in the sugar cane plant depends upon the fact that many foods must be digested before they can be translocated, and enzymes are essential for the process of digestion. The im-

portance of enzymes in the growth and nutrition of plants has already been discussed elsewhere (14). Insoluble foods such as starch and other polysaccharides cannot move from place to place in the plant without first being changed to soluble forms, and this change requires the aid of specific enzymes. An illustration of the importance of enzymes in translocation may be taken from a hitherto unpublished experiment in which the activity of the enzyme amylase (starch-splitting) in the growing point of Uba cane was found to be weaker than that of six other varieties of sugar cane, growing side by side. It is suggested that the weaker activity of amylase in the growing point of Uba cane may aid in explaining the failure of starch to disappear from the stalk of that variety of sugar cane. With more active amylase the insoluble starch could be digested to a soluble sugar and be used in translocation, storage, or growth.

The importance of invertase in aiding the translocation of sugar has already been mentioned. Because the ratio between cane sugar and the simple sugars in the sheaths remains nearly constant during the day and the night, it is suggested that when some factor causes a decided increase or decrease in one kind of sugar, equilibrium is reached again quickly due to the active invertase which has been found to be of general occurrence in the sheaths of the sugar cane plant.

The necessity for a supply of sugar for translocation may be taken as axiomatic. The more sugar there is made in the leaf the more there is available for translocation, unless some other factor becomes limiting. Thus it is only natural that during a cold, rainy, cloudy season less sugar is translocated to the stems than during a warm sunny period, because under the former conditions the formation of sugar is diminished on account of the decreased rate of photosynthesis. Of course other processes besides photosynthesis and translocation are affected by such factors as temperature, rain, etc., and in the final analysis, the amount of sugar available for translocation from the blades is the surplus over that used within the blades.

What has just been said for sugar in general would seem to apply to the translocation of specific sugars. It has been suggested above that in the December experiment cane sugar was translocated into the sheaths as long as there was a supply available in the blades, and that during the night the simple sugars were translocated more than sucrose merely because the hydrolysis of polysaccharides in the blades during the night furnished a greater supply of simple sugars than of cane sugar for translocation.

Thus there are two main sources of supply of sugar for translocation: photosynthesis, which is limited to the daylight hours; and the digestion of polysaccharides, which occurs chiefly during the night.

The formation of polysaccharides in the blades occurs during the day as a secondary result of photosynthesis. The manufacture of the insoluble starch and other insoluble polysaccharides removes considerable quantities of soluble sugars from solution. This is important inasmuch as the accumulation of all the soluble sugars formed in photosynthesis might interfere with that process seriously since the accumulation of the end products of a chemical reaction is known to decrease the speed of that reaction. The temporary storage of polysaccharides in the blades during the day aids in translocation by furnishing a supply of sugars for translocation after nightfall, when photosynthesis stops. This suggestion is based upon the

assumption that it is anatomically and physiologically impossible for the phloem of the leaves to remove all of the photosynthate as fast as it is formed.

When the supply of sugars entering the sheaths is greater than that which is passing on immediately into the stems, the conditions are ripe for the formation of polysaccharides in the sheaths. Evidence of such a formation occurring in both the April and December experiments has been given in Figs. 1 and 3. In the April experiment, polysaccharides reached a maximum in the sheaths at the time of their minimum concentration in the blades, at 11 p.m. to 3 a.m., indicating that the removal of polysaccharides from the blades by digestion and translocation led to their formation in the sheaths. This was followed by the digestion of polysaccharides and formation of simple sugars in the sheaths, a process necessary for translocation to the stems. Similarly in the December experiment polysaccharides remained high in concentration in the sheaths several hours after they had decreased in the blades, and their sharp decline in the sheaths was accompanied by a marked increase in simple sugars. The evidence indicates that a temporary storage of polysaccharides in the sheaths during the night aids in the translocation of carbohydrates from the blades to the stems.

Another factor affecting translocation is the water content. In the April experiment the sheaths contained more sucrose than simple sugars at every hour tested, whereas in the December experiment the sheaths contained less sucrose than simple sugars. The plants used in the two experiments differed in moisture content. The younger plants taken in December had higher moisture percentages than the older plants taken in April. The lower temperatures and greater amount of cloudiness in December than in April would naturally decrease the rate of water loss from the leaves of the plants taken in December. Differences in age and in the rate of loss of water were probably more important than rainfall in causing the differences in water content, because differences in rainfall were compensated for by irrigation. Therefore it would seem that the immediate cause of the relative amounts of the sugars in the sheaths was the difference in moisture percentage, which in turn was due to the differences in age and season. A similar condition was found in another experiment in plants of the same age which received different supplies of water (12). The sheaths of the plants deprived of water contained more sucrose than simple sugar, whereas the sheaths of the plants supplied with water had more simple sugar than cane sugar. Another experiment not yet published (13) also shows that the factors which increase the moisture percentage favor digestion by invertase rather than synthesis. It is suggested that the relative amounts of the sugars in the sheaths are affected by the amount of water in the sheaths and that the translocation form of sugar predominant in a given plant at a given time is largely dependent upon the content of water.

Without doubt factors other than those just mentioned have important effects upon the process of translocation in the sugar cane plant. The sugar contained in the juices of cane has all originated in the leaf. Studies of the conditions favoring or hindering the translocation of carbohydrates from the leaf to the stalk of sugar cane are essential for an understanding of the fundamentals of sugar production and may lead to an advance in our knowledge of the ripening of cane.

## SUMMARY

1. This paper introduces the subject of translocation in the sugar cane plant by a consideration of the nature of the process, the location of its occurrence, the time it takes place, the external and internal factors affecting its speed, and the theories regarding the mechanism of translocation.

2. The results of the analyses of moisture, simple sugars, cane sugar, starch, and total polysaccharides in the sheaths taken at hourly or bi-hourly intervals during the day and the night, are presented in tabular and graphic form.

3. The transport of sugar is shown to occur both day and night.

4. Both cane sugar and the simple sugars are translocated in the sugar cane plant.

5. An important factor determining which form of sugar is translocated the more predominantly in a given plant at a given time is the percentage of water.

6. The temporary storage of polysaccharides in the sheath during the night aids in the translocation of carbohydrates from the blade to the stem.

## ACKNOWLEDGMENT

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## Further Notes on Water and Cane Ripening

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By CONSTANCE E. HARTT

The results of a study of the effects of water upon the ripening of sugar cane were reported in 1934 (5). The evidence presented then indicated that some photosynthesis occurred at the wilting point, and that a greater synthesis of sucrose took place in the blades of sugar cane plants supplied with water than in those deprived of water. The first experiment was merely preliminary and the interpretation of the differences was not entirely clear. The study was therefore repeated, and this report touches upon certain aspects of the effect of water upon photosynthesis, translocation, and storage of sugar in the sugar cane plant.

At the suggestion of W. W. G. Moir, the second experiment was expanded to include studies of the enzyme activity in the plants undergoing treatment. Since enzymes are sensitive to external and internal conditions of many kinds, and since other experiments have indicated that they are affected by water content (6), and may thus control the formation of starch and sugar (3, 4), it was hoped that a study of their activity might aid in elucidating the problems of water and cane ripening.

Brief definitions of photosynthesis and of the carbohydrates (8) mentioned in this report, of translocation (9), and of enzymes (7) have been presented in other reports.

### METHODS

Sugar cane of the variety H 109 was planted in pots of good soil on October 8, 1934, and kept in a sunny part of the grounds of the Experiment Station. The plants were watered and fertilized through the kindness of Dr. A. J. Mangelsdorf, all of the plants receiving optimum water and fertilizer applications until the beginning of the experiment.

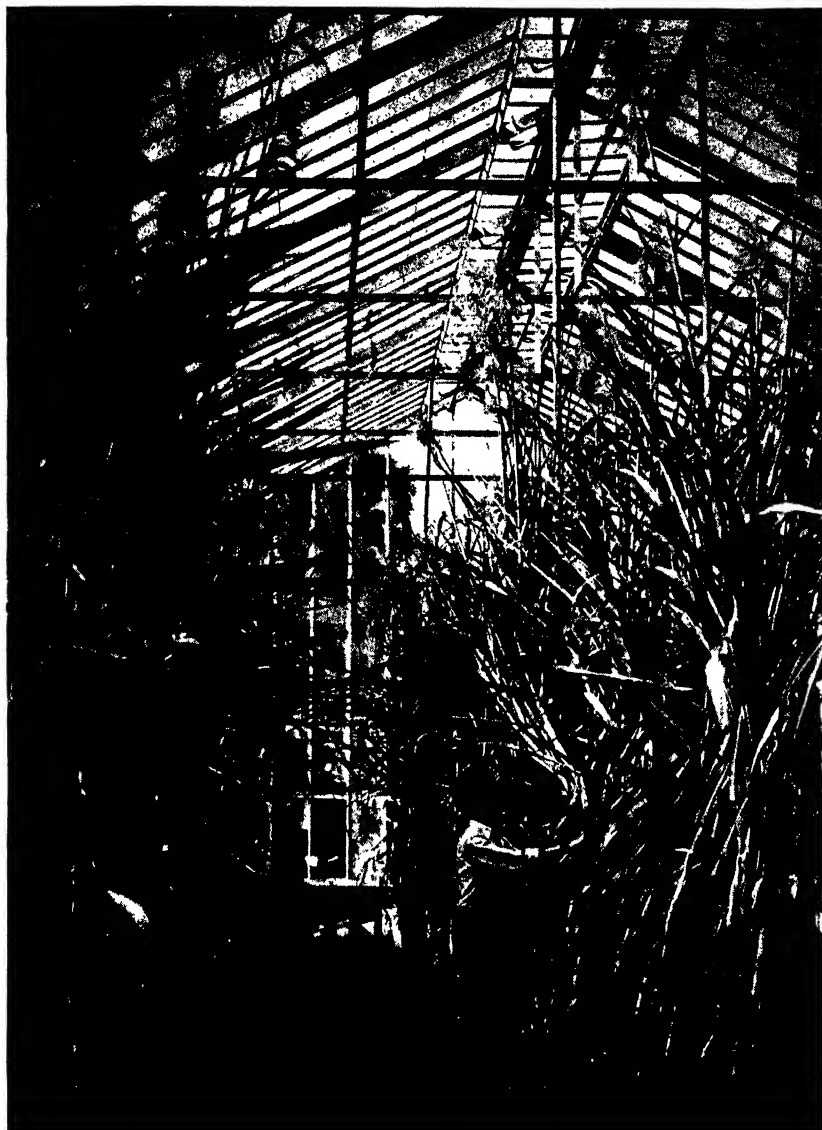
The plants were allowed to grow until November 15, 1935, at which time most of the stalks were tasseling. The following series of plants were then inaugurated:

1. Dark wet
2. Dark dry
3. Light wet
4. Light dry
5. Outdoor control

There were 15 pots in each series, the plants in each series being taken from all parts of the plot.

Series 5, the outdoor controls, were left in their original places throughout the experiment, and were watered daily.

On Friday, November 15, 1935, when the plants were 13 months and one week of age, series 1-4 were removed to the greenhouse. Beginning Saturday, November 16, the "dry" plants (series 2 and 4) received no further water, while the "wet" plants (series 1 and 3) were watered daily. By November 20, the "dry" plants



**Fig. 1.** Sugar cane plants of the variety II 109: at the right, water withheld for five days; at the left, water supplied daily. Photographed the day of removal to the dark.

appeared to be wilting, the leaves being dry, yellow, and somewhat curled at noon. The "wet" plants were in good condition. The appearance of the plants in the greenhouse on November 20 is illustrated in Fig. 1. All of the plants in series 1-4 were placed in the darkened assembly room beginning at 5 p. m. The temperature in the lecture hall was approximately 80-83° F. all of the following day, the heating of the room being conducted with the efficient cooperation of W. Sa Ning. The "light" plants (series 3 and 4) were removed from the darkened assembly room to the greenhouse on the evening of November 21, and received light the following day until 1 p. m., when they were harvested. The "dark" plants remained in the dark until 8 a. m. November 22, when they were harvested. The "outdoor control" plants were harvested at 1 p. m.

The purpose of putting the plants in the dark was to use up their stored carbohydrates. The plants were returned to the light to allow them to conduct photosynthesis.

The following samples were taken in each series, using only tasseling cane:

Blades: Nos. 5, 6, 7, 8 (unless very yellow), midribs included.

Sheaths: Nos. 5, 6, 7, 8.

Green-leaf cane: between attachment of leaf No. 5 and of the lowest living leaf.

Upper dry-leaf cane: the upper half.

Lower dry-leaf cane: the lower half.

Samples were taken for moisture, sugars, and polysaccharides, which were analyzed by our regular methods, the references to which were cited in the former paper (5). The upper and lower dry-leaf cane were sampled for juice analyses, which were conducted by the Sugar Technology Department. The material for the study of enzyme activity was dried with the drying apparatus recently perfected under the direction of Dr. H. L. Lyon, which dries the ground plant material at a temperature between 30 and 40° C.

The enzymes studied in this investigation included invertase, amylase, dextrinase, and maltase. The activity of invertase, the enzyme which aids in the digestion of cane sugar forming simple sugars (glucose and fructose), was determined by measuring the increase in the ability to reduce Soxhlet's solution, when weighed amounts of plant powder (containing the enzyme) were incubated for 24 hours with known quantities of pure sucrose solution as the substrate. The activity of maltase, an enzyme which aids in the digestion of maltose forming glucose, was measured in the same way, using known quantities of pure maltose solution as the substrate. Sucrose does not reduce Soxhlet's solution, so that after a period of incubation any reducing action found is due to the presence of simple sugars, which do reduce Soxhlet's solution. Maltose also reduces Soxhlet's solution, but its reducing action is not as great as that of glucose; hence after a period of incubation any increase in reducing action is due to the presence of glucose. The tests were all performed in duplicate, with suitable controls, and were repeated. The reducing action was determined by the method of Munson and Walker (10). The Bertrand (1) method, titration with potassium permanganate, was used for estimating the amount of copper oxide formed in the Soxhlet's solution. The results are expressed in cubic

centimeters of twentieth normal potassium permanganate, the higher the figure the greater the activity of the enzyme.

The activity of amylase, the enzyme which aids in the digestion of starch forming dextrine, is given as color with iodine-potassium iodide, after incubation for 20 hours at 36.5° C., using soluble starch as the medium. The activity of dextrinase, the enzyme which aids in the digestion of dextrine forming maltose, is given similarly, using dextrine as the medium. The following five colors are used to represent the course of the digestion of starch and dextrine, in the order named: blue, purple, red-purple, dark red, red.

Soil samples for the determination of soil moisture were taken by H. A. Wadsworth, who reported that at the time of the experiment both the "dark dry" and the "light dry" (series 2 and 4) were below the wilting percentage. The wilting percentage, or soil moisture content at which plants wilt, is considered a critical point by Veihmeyer and Hendrickson (13), who found that the leaves of plants below the wilting point had narrower openings of the stomatal pores than the leaves of plants supplied with water; and that no differences in stomatal opening were noticeable in plants above the wilting point even though the soil moisture content fluctuated.

On November 23 the plants were ratooned and irrigation and fertilization started in the usual way. To determine whether or not the drying of the plants affected the development of the ratoons, counts were made of the number of pots which had developed tillers by December 3. The total number of pots which had previously been deprived of water was 28, and all of these contained tillers. The total number of pots which had received water throughout the experiment was 44, but only 40 of these contained tillers on December 3. On December 16 a count was made of the number of tillers per pot, and it was found that the "dry" varied from two to 14, with an average of six, whereas the "wet" varied from one to nine, with an average of five. Evidently the plants previously deprived of water commenced development as well if not better than the plants adequately supplied with water.

The ratoons are now growing and it is planned to repeat the experiment before the development of tassels. It is then hoped to undertake a broader program covering the conditions for ripening for early, intermediate, and late harvest.

## RESULTS

The results of the juice analyses are presented in Table I, which shows that the "dark wet" was superior to the "dark dry," and the "light wet" was superior to the "light dry" in the upper dry-leaf cane. In the lower dry-leaf cane, the "dark wet" was superior to the "dark dry," but the "light wet" was not superior to the "light dry."

The results of the moisture determinations are reported in Table II, in which the results are expressed both upon the wet-weight basis and upon the dry-weight basis. These results are disappointing inasmuch as the "dry" series had higher percentages of moisture than the "wet" series, in several places. These results are the moisture percentages of the samples and may not be a true indication of the moisture contents of the plants themselves. In this experiment such large plants and large amounts of material had to be handled that a longer time than usual elapsed between cutting the stalks and weighing the ground samples. In the first

experiment, the "wet" plants had higher percentages of moisture than the "dry" plants in every organ tested.

The percentages of simple sugars are presented in Table III, cane sugar in Table IV, polysaccharides in Table V, and starch in Table VI. The results are expressed in three ways: in percentages upon the wet-weight basis, the dry-weight basis, and the residual dry-weight basis. The last method, which is recommended for experiments of short duration, will be used as the chief basis for discussion in this report. The residual dry weight is the difference between the dry weight and the total sugars plus polysaccharides and is considered to form a more reliable basis for comparison than either the wet-weight basis or the dry-weight basis because it rules out the greater part of the fluctuating compounds.

The results of the carbohydrate analyses in the blades and the sheaths, expressed upon the residual dry-weight basis, are presented in graphic form in Fig. 2, those in the green-leaf cane in Fig. 3, those in the upper dry-leaf cane in Fig. 4, and those in the lower dry-leaf cane in Fig. 5. In Fig. 6 are presented the graphs of the total sugars plus polysaccharides, which may be taken to represent the total metabolizable carbohydrate material. The possible relationships of these results will be considered in the discussion.

The activity of invertase, determined without additional buffers and at pH 4.5 using the buffers of McIlvaine (2), is reported in Table VII. The results of the determinations of maltase activity are presented in Table VIII, and those of amylase and dextrinase in Table IX.

TABLE I

## Juice Analyses

Series	Brix	Pol.	Sucrose	Apparent Purity	Gravity Purity	Glucose	Q.R.
Upper dry-leaf cane							
1—dark wet	18.07	14.88	15.34	82.35	84.89	1.51	9.34
2—dark dry	17.54	14.25	14.72	81.24	83.92	1.58	9.85
3—light wet	18.23	15.34	15.80	84.15	86.73	1.33	8.93
4—light dry	17.83	14.47	14.98	81.16	84.02	1.60	9.71
5—outdoor control	18.42	14.81	15.34	80.40	83.28	1.67	9.55
Lower dry-leaf cane							
1—dark wet	20.27	19.19	19.45	94.67	95.95	0.15	6.64
2—dark dry	19.82	18.60	18.81	93.84	94.90	0.23	6.88
3—light wet	20.02	18.60	18.91	92.91	94.46	0.26	6.92
4—light dry	19.87	18.73	18.94	94.26	95.32	0.13	6.82
5—outdoor control	20.50	19.04	19.37	92.88	94.49	0.28	6.76

TABLE II

## Moisture Determinations

Series	Blades	Sheaths	Green cane	Upper dry	Lower dry
Percentages expressed on wet-weight basis					
1—dark wet	64.75±0.029	66.18	79.09±0.009	73.07±0.038	66.93±0.024
2—dark dry	65.61±0.048	68.08±0.019	79.30±0.052	74.12±0.019	68.59±0.052
3—light wet	64.25±0.086	69.60±0.286	77.51±0.186	70.77±0.172	70.06±0.062
4—light dry	63.88±0.176	65.70	78.84±0.114	73.57±0.133	69.99±0.024
5—outdoor control	65.06±0.048	69.95±0.229	78.45±0.014	72.20±0.162	68.41±0.114
Percentages expressed on dry-weight basis					
1—dark wet	183.7±0.238	195.7	378.3±0.191	271.3±0.525	202.4±0.238
2—dark dry	190.8±0.382	213.3±0.191	383.1±1.240	286.4±0.286	218.4±0.525
3—light wet	179.7±0.668	229.1±3.100	344.9±3.673	242.1±2.003	234.0±0.715
4—light dry	176.9±1.384	191.5	372.7±2.576	278.4±1.908	233.2±0.238
5—outdoor control	186.2±0.382	232.8±2.528	349.3±7.441	259.8±2.146	216.5±1.097

TABLE III

Simple sugars; percentages expressed on the wet-weight basis, the dry-weight basis and the residual dry-weight basis.

Series	Wet weight	Dry weight	Residual dry weight
Blades:			
1—dark wet .....	0.200±0.006	0.566±0.016	0.790±0.021
2—dark dry .....	0.437±0.017	1.273±0.051	1.814±0.077
3—light wet .....	0.316±0.000	0.884±0.001	1.297±0.002
4—light dry .....	0.550±0.001	1.525±0.001	2.199±0.006
5—outdoor control .....	0.339±0.001	0.972±0.005	1.410±0.007
Sheaths:			
1—dark wet .....	0.696±0.001	2.059±0.004	2.978±0.008
2—dark dry .....	0.822±0.007	2.577±0.023	3.741±0.042
3—light wet .....	0.740±0.001	2.435±0.003	3.679±0.015
4—light dry .....	0.855±0.004	2.495±0.013	3.720±0.011
5—outdoor control .....	0.860±0.005	2.864±0.004	4.361
Green-leaf cane:			
1—dark wet .....	2.157±0.016	10.321±0.079	28.912±0.241
2—dark dry .....	2.064±0.007	9.972±0.032	27.808±0.128
3—light wet .....	1.948±0.006	8.664±0.028	22.987±0.138
4—light dry .....	2.389±0.002	11.292±0.011	33.104
5—outdoor control .....	1.976±0.004	9.233±0.013	25.730
Upper dry-leaf cane:			
1—dark wet .....	1.421±0.003	5.276±0.012	17.301±0.168
2—dark dry .....	1.475±0.008	5.701±0.029	23.109±0.178
3—light wet .....	0.679±0.010	2.324±0.034	7.440±0.101
4—light dry .....	1.122±0.017	4.249±0.066	16.329±0.213
5—outdoor control .....	1.131±0.005	4.069±0.019	13.144±0.054
Lower dry-leaf cane:			
1—dark wet .....	0.404±0.007	1.223±0.021	3.787±0.085
2—dark dry .....	0.272±0.002	0.867±0.008	3.195±0.010
3—light wet .....	0.328±0.002	1.097±0.007	3.952±0.026
4—light dry .....	0.279±0.002	0.932±0.004	3.478±0.012
5—outdoor control .....	0.334±0.005	1.059±0.015	3.060±0.028

TABLE IV

Cane sugar; percentages expressed on the wet-weight basis, the dry-weight basis,  
and the residual dry-weight basis.

Series	Wet weight	Dry weight	Residual dry weight
<b>Blades:</b>			
1—dark wet .....	$0.509 \pm 0.002$	$1.447 \pm 0.019$	$2.016 \pm 0.023$
2—dark dry .....	$0.585 \pm 0.019$	$1.700 \pm 0.054$	$2.421 \pm 0.083$
3—light wet .....	$1.435 \pm 0.011$	$4.016 \pm 0.032$	$5.894 \pm 0.049$
4—light dry .....	$1.071 \pm 0.004$	$2.966 \pm 0.025$	$4.275 \pm 0.019$
5—outdoor control .....	$1.225 \pm 0.022$	$3.506 \pm 0.062$	$5.085 \pm 0.088$
<b>Sheaths:</b>			
1—dark wet .....	$1.412 \pm 0.039$	$4.176 \pm 0.115$	$6.039 \pm 0.169$
2—dark dry .....	$1.226 \pm 0.010$	$3.840 \pm 0.033$	$5.573 \pm 0.062$
3—light wet .....	$1.373 \pm 0.006$	$4.516 \pm 0.020$	$6.823 \pm 0.066$
4—light dry .....	$1.456 \pm 0.003$	$4.245 \pm 0.008$	$6.328 \pm 0.000$
5—outdoor control .....	$2.087 \pm 0.011$	$6.946 \pm 0.030$	10.313
<b>Green-leaf cane:</b>			
1—dark wet .....	$6.772 \pm 0.036$	$32.383 \pm 0.182$	$90.707 \pm 0.574$
2—dark dry .....	$6.388 \pm 0.026$	$30.861 \pm 0.125$	$86.057 \pm 0.471$
3—light wet .....	$6.463 \pm 0.001$	$28.735 \pm 0.004$	$76.223 \pm 0.225$
4—light dry .....	$6.443 \pm 0.020$	$30.449 \pm 0.094$	89.646
5—outdoor control .....	$7.438 \pm 0.007$	$34.457 \pm 0.003$	103.93
<b>Upper dry-leaf cane:</b>			
1—dark wet .....	$11.646 \pm 0.045$	$43.298 \pm 0.190$	$141.813 \pm 1.594$
2—dark dry .....	$11.457 \pm 0.034$	$44.271 \pm 0.131$	$176.831 \pm 1.782$
3—light wet .....	$12.224 \pm 0.046$	$41.822 \pm 0.158$	$133.859 \pm 0.668$
4—light dry .....	$11.787 \pm 0.025$	$44.598 \pm 0.096$	$171.395 \pm 0.788$
5—outdoor control .....	$11.150 \pm 0.078$	$40.110 \pm 0.283$	$129.621 \pm 2.066$
<b>Lower dry-leaf cane:</b>			
1—dark wet .....	$14.214 \pm 0.031$	$42.985 \pm 0.097$	$132.977 \pm 0.322$
2—dark dry .....	$14.730 \pm 0.043$	$46.898 \pm 0.139$	$172.770 \pm 1.429$
3—light wet .....	$14.254 \pm 0.013$	$47.608 \pm 0.045$	$171.347 \pm 0.174$
4—light dry .....	$14.570 \pm 0.065$	$48.550 \pm 0.216$	$181.152 \pm 1.920$
5—outdoor control .....	$13.393 \pm 0.115$	$42.395 \pm 0.364$	$122.519 \pm 1.635$



TABLE V

Polysaccharides; percentages expressed on the wet-weight basis, the dry-weight basis, and the residual dry-weight basis.

Series	Wet weight	Dry weight	Residual dry weight
<b>Blades:</b>			
1—dark wet .....	9.14±0.095	25.96±0.277	36.21±0.439
2—dark dry .....	9.20±0.024	26.77±0.062	38.14±0.195
3—light wet .....	9.62±0.005	26.95±0.019	39.57±0.029
4—light dry .....	9.43±0.105	26.14±0.300	37.72±0.587
5—outdoor control .....	9.29±0.029	26.59±0.086	38.61±0.133
<b>Sheaths:</b>			
1—dark wet .....	8.31±0.029	24.61±0.086	35.61±0.100
2—dark dry .....	7.88±0.038	24.68±0.119	35.84±0.257
3—light wet .....	8.15±0.100	26.84±0.334	40.59±0.725
4—light dry .....	8.93±0.048	26.18±0.148	39.05±0.296
5—outdoor control .....	7.12	23.67	35.59
<b>Green-leaf cane:</b>			
1—dark wet .....	4.51±0.048	21.59±0.234	60.56±0.620
2—dark dry .....	4.82±0.019	23.30±0.105	65.09±0.215
3—light wet .....	5.59±0.014	24.90±0.071	66.15±0.405
4—light dry .....	5.05	23.85	69.94
5—outdoor control .....	4.43	20.57	57.61
<b>Upper dry-leaf cane:</b>			
1—dark wet .....	6.81±0.038	25.28±0.148	83.06±1.078
2—dark dry .....	6.56±0.024	25.35±0.095	102.85±0.095
3—light wet .....	7.19±0.024	24.61±0.086	78.87±0.153
4—light dry .....	6.65±0.009	25.13±0.033	96.84±0.396
5—outdoor control .....	6.91±0.000	24.90±0.009	80.62±0.706
<b>Lower dry-leaf cane:</b>			
1—dark wet .....	7.77±0.076	23.46±0.224	72.66±1.021
2—dark dry .....	7.89±0.005	25.08±0.009	92.57±0.534
3—light wet .....	7.04±0.009	23.51±0.033	84.87±0.119
4—light dry .....	7.12±0.014	23.71±0.043	88.64±0.391
5—outdoor control .....	6.94±0.057	21.93±0.181	63.48±0.224

TABLE VI

Starch; percentages expressed on the wet-weight basis, the dry-weight basis,  
and the residual dry-weight basis.

Series	Wet weight	Dry weight	Residual dry weight
<b>Blades:</b>			
1—dark wet .....	$0.188 \pm 0.022$	$0.534 \pm 0.062$	$0.745 \pm 0.088$
2—dark dry .....	$0.080 \pm 0.019$	$0.235 \pm 0.055$	$0.335 \pm 0.079$
3—light wet .....	$0.181 \pm 0.009$	$0.508 \pm 0.025$	$0.745 \pm 0.037$
4—light dry .....	$0.058 \pm 0.005$	$0.161 \pm 0.013$	$0.232 \pm 0.020$
5—outdoor control .....	$0.128 \pm 0.003$	$0.367 \pm 0.008$	$0.533 \pm 0.012$
<b>Sheaths:</b>			
1—dark wet .....	$0.055 \pm 0.005$	$0.165 \pm 0.016$	$0.239 \pm 0.024$
2—dark dry .....	$0.066 \pm 0.000$	$0.207 \pm 0.001$	$0.301 \pm 0.001$
3—light wet .....	$0.073 \pm 0.006$	$0.240 \pm 0.019$	$0.363 \pm 0.031$
4—light dry .....	$0.060 \pm 0.005$	$0.177 \pm 0.015$	$0.264 \pm 0.023$
5—outdoor control .....	$0.038 \pm 0.002$	$0.130 \pm 0.007$	0.172
<b>Green-leaf cane:</b>			
1—dark wet .....	0.659	3.154	8.859
2—dark dry .....	$0.462 \pm 0.000$	$2.233 \pm 0.004$	$6.238 \pm 0.003$
3—light wet .....	$0.481 \pm 0.010$	$2.141 \pm 0.048$	$5.689 \pm 0.145$
4—light dry .....	0.408	1.927	5.651
5—outdoor control .....	$0.677 \pm 0.017$	$3.145 \pm 0.088$	8.294
<b>Upper dry-leaf cane:</b>			
1—dark wet .....	$0.913 \pm 0.010$	$3.390 \pm 0.038$	$11.139 \pm 0.202$
2—dark dry .....	$0.841 \pm 0.033$	$3.249 \pm 0.129$	$13.189 \pm 0.563$
3—light wet .....	$1.242 \pm 0.016$	$4.248 \pm 0.054$	$13.615 \pm 0.153$
4—light dry .....	$1.112 \pm 0.044$	$4.204 \pm 0.167$	$16.194 \pm 0.599$
5—outdoor control .....	$1.289 \pm 0.021$	$4.642 \pm 0.075$	$15.019 \pm 0.104$
<b>Lower dry-leaf cane:</b>			
1—dark wet .....	$1.238 \pm 0.023$	$3.740 \pm 0.071$	$11.577 \pm 0.168$
2—dark dry .....	$1.031 \pm 0.002$	$3.280 \pm 0.010$	$12.105 \pm 0.026$
3—light wet .....	$0.963 \pm 0.034$	$3.153 \pm 0.083$	$11.384 \pm 0.302$
4—light dry .....	$0.949 \pm 0.044$	$3.162 \pm 0.146$	$11.839 \pm 0.620$
5—outdoor control .....	$1.383 \pm 0.001$	$4.371 \pm 0.004$	$12.652 \pm 0.073$

TABLE VII

Invertase activity expressed in cc. N/20 KMnO<sub>4</sub>.

Series	Blades	Sheaths	Green cane	Upper dry	Lower dry
Activity Unbuffered					
1—dark wet .....	6.92	10.56	3.61	1.67	1.42
2—dark dry .....	7.64	9.94	6.70	1.45	1.09
3—light wet .....	9.08	10.33	6.07	1.33	0.67
4—light dry .....	6.71	12.95	6.06	2.35	0.58
5—outdoor control ....	17.34	14.92	5.92	2.57	2.01
Activity at pH 4.5.					
1—dark wet .....	10.65	15.90	5.15	2.07	1.48
2—dark dry .....	12.22	15.19	5.97	2.22	1.78
3—light wet .....	15.48	18.05	5.83	1.14	1.66
4—light dry .....	12.10	18.94	5.70	2.36	1.44
5—outdoor control ....	20.72	17.02	5.11	2.34	2.74

TABLE VIII

Maltase activity expressed in cc. N/20 KMnO<sub>4</sub>.

Series	Blades	Sheaths	Green cane	Upper dry	Lower dry
Activity Unbuffered					
1—dark wet .....	3.35	5.21	7.96	4.51	4.07
2—dark dry .....	3.81	3.95	13.36	3.61	2.53
3—light wet .....	6.44	5.11	12.61	4.72	3.56
4—light dry .....	4.16	3.62	14.57	5.27	2.18
5—outdoor control ....	4.11	4.86	11.78	6.61	6.46
Activity at pH 4.5.					
1—dark wet .....	3.72	5.25	7.49	6.20	5.15
2—dark dry .....	5.42	3.87	7.60	7.01	4.72
3—light wet .....	6.47	3.47	7.64	3.50	3.55
4—light dry .....	4.40	2.71	9.02	6.90	4.07
5—outdoor control ....	4.30	3.49	7.60	7.55	5.92

TABLE IX

Amylase and dextrinase activity, expressed as color when tested with iodine.

Series	Amylase	Dextrinase
<b>Blades:</b>		
1—dark wet .....	dark red	dark red
2—dark dry .....	red-purple	red-purple
3—light wet .....	red	dark red
4—light dry .....	red-purple	red-purple
5—outdoor control .....	red	dark red
<b>Sheaths:</b>		
1—dark wet .....	purple	red-purple
2—dark dry .....	purple	red-purple
3—light wet .....	purple	red-purple
4—light dry .....	purple	red-purple
5—outdoor control .....	purple	red-purple
<b>Green-leaf cane:</b>		
1—dark wet .....	blue	purple
2—dark dry .....	blue	purple
3—light wet .....	blue	purple
4—light dry .....	blue	purple
5—outdoor control .....	blue	purple
<b>Upper dry-leaf cane:</b>		
1—dark wet .....	blue	purple
2—dark dry .....	blue	purple
3—light wet .....	blue	purple
4—light dry .....	blue	purple
5—outdoor control .....	blue	purple
<b>Lower dry-leaf cane:</b>		
1—dark wet .....	blue	purple
2—dark dry .....	blue	purple
3—light wet .....	blue	purple
4—light dry .....	blue	purple
5—outdoor control .....	blue	purple

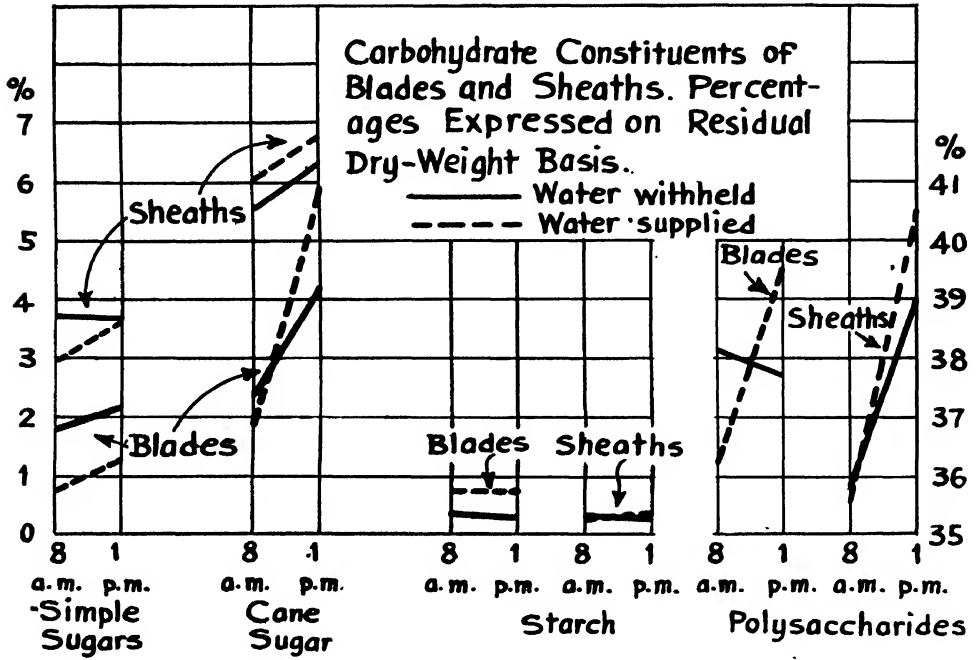


Fig. 2.

# Carbohydrate Constituents of Green-Leaf Cane. Percentages Expressed on Residual Dry-Weight Basis.

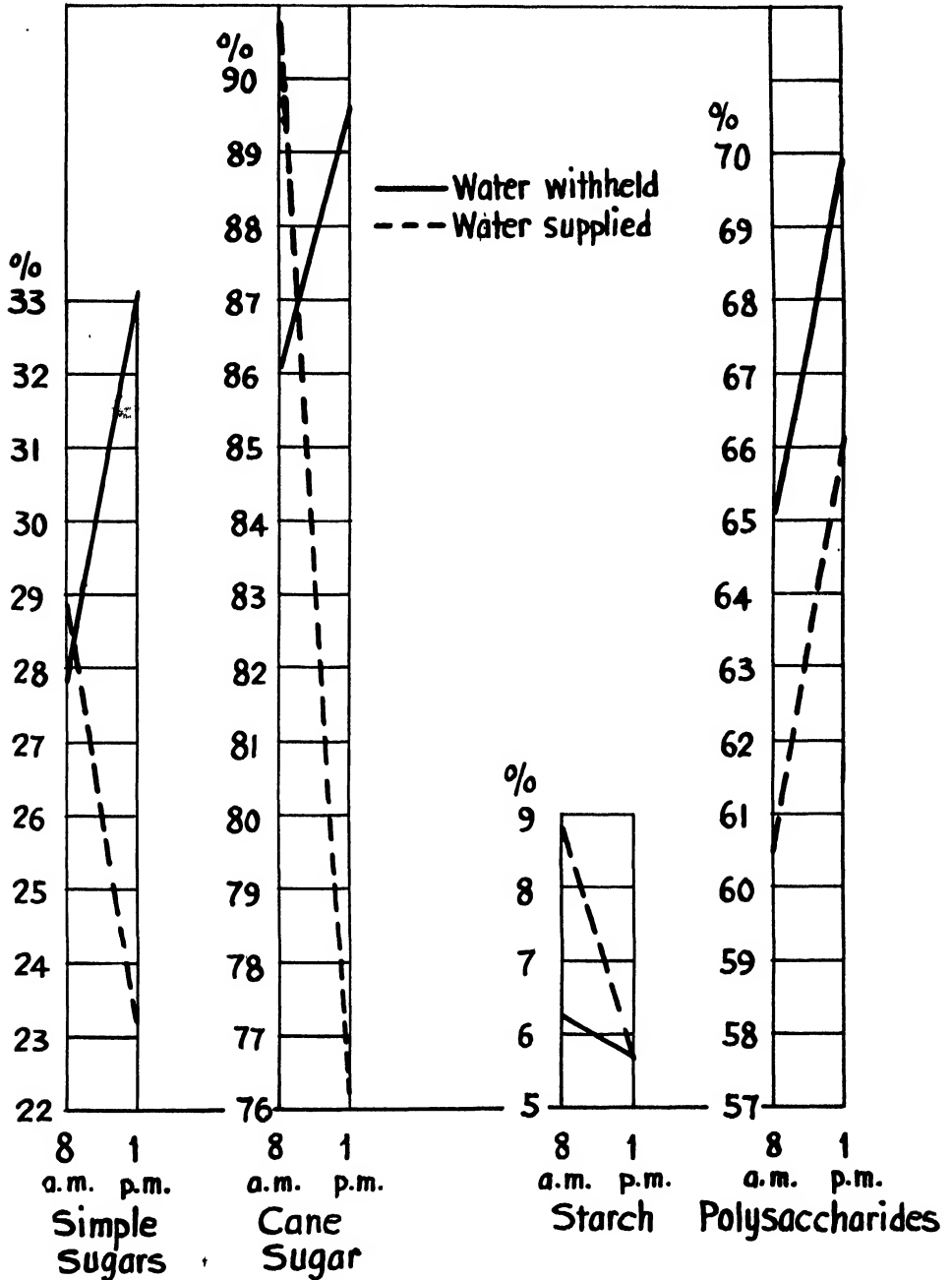


Fig. 3.

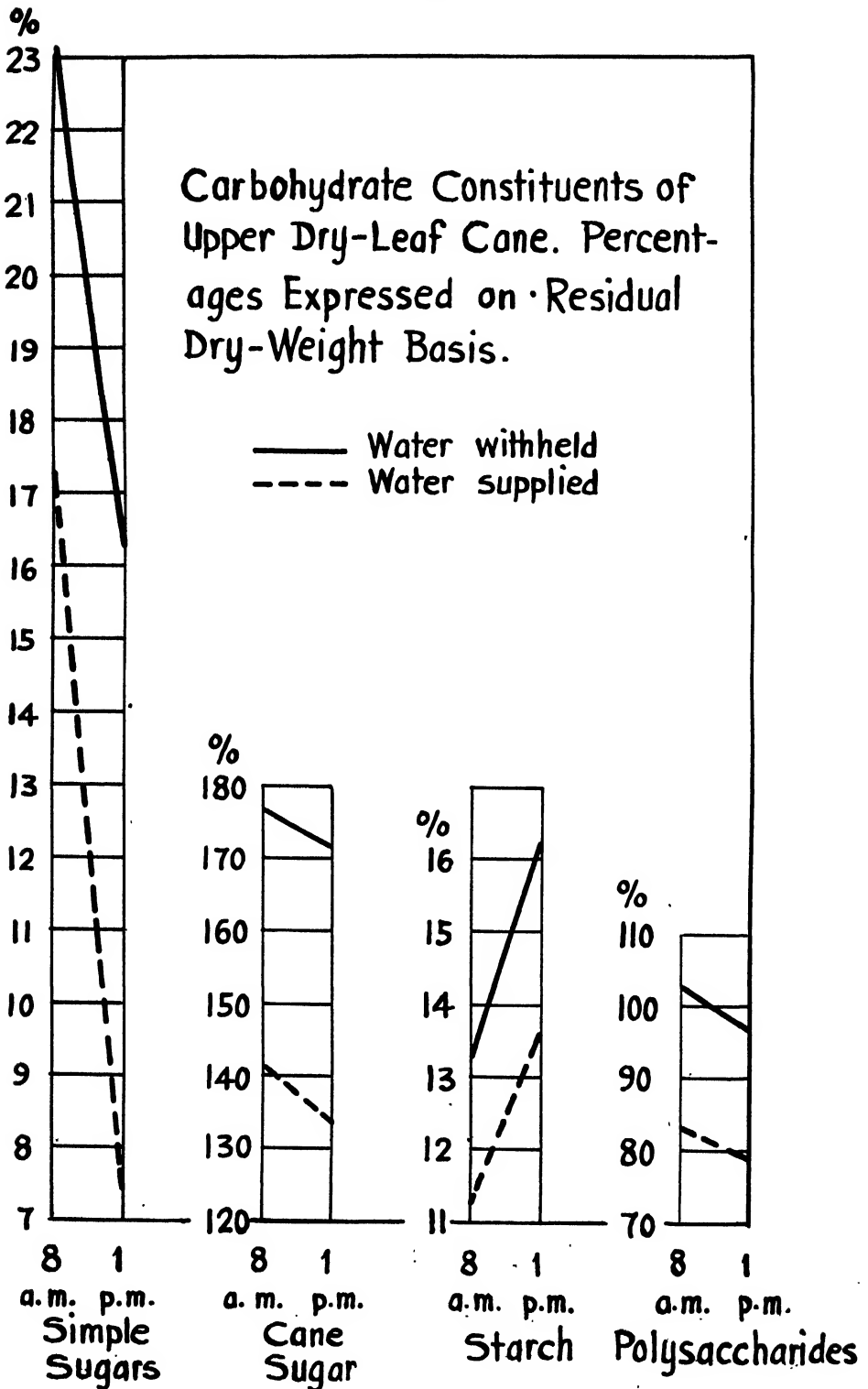


Fig. 4.

**Carbohydrate Constituents  
of Lower Dry-Leaf Cane.  
Percentages Expressed on  
Residual Dry-Weight Basis.**

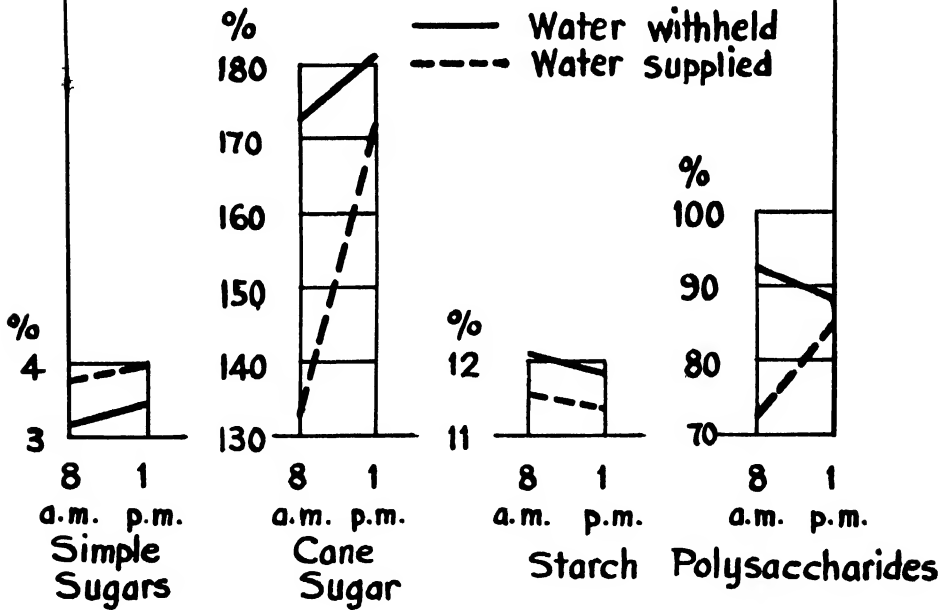


Fig. 5.



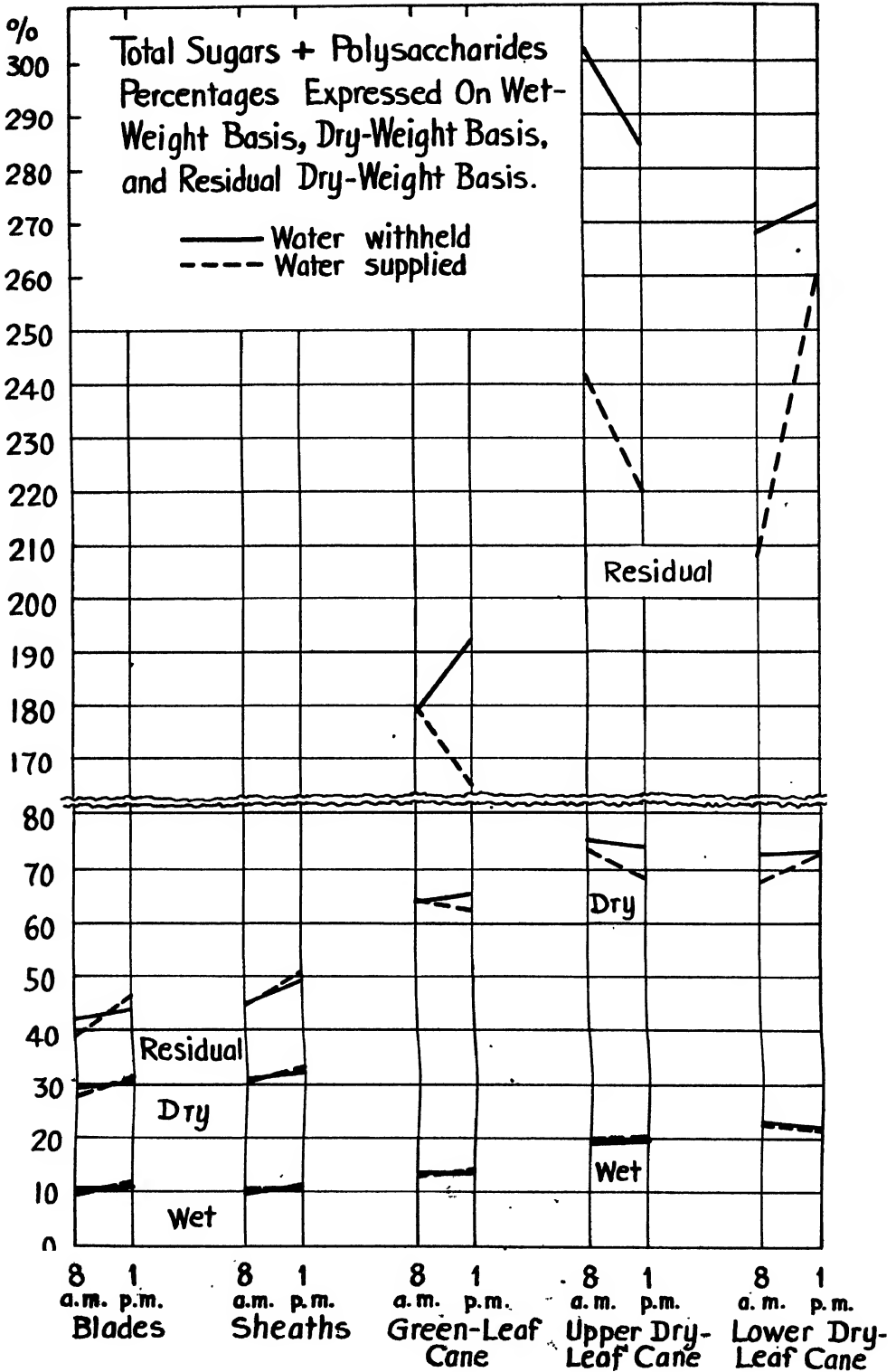


Fig. 6.

## DISCUSSION

*Simple Sugars:*

The fluctuations in the simple or reducing sugars (glucose and fructose) are in the same direction on the three bases of calculation with the exception of the lower dry-leaf cane, in which the plants supplied with water decreased slightly during exposure to light when calculated upon the wet and dry bases but increased a trifle when calculated upon the residual dry-weight basis. The differences are accentuated upon the residual basis, and from these results the following observations are recorded:

In the blades, the content of simple sugars increased during exposure to light, in both series of plants, with a greater increase in the plants supplied with water than in those deprived of water. Thus although the manufacture of simple sugars may take place in the blades of plants below the wilting point, yet the process proceeds better in plants receiving an adequate supply of water.

In the sheaths there was an increase in simple sugars in the plants supplied with water but not in the others. It may be either that the favorable water supply increased the production of simple sugars in the sheaths, or that the sheaths of the plants supplied with water received more simple sugars from the blades, because the blades were able to manufacture more simple sugars.

In the green-leaf cane, there was a decrease in the percentage of simple sugars in the plants receiving water, but an increase in the plants deprived of water. These differences seem best explained, at least in part, by utilization in growth and respiration in the plants receiving water and a curtailment of such utilization in the plants deprived of water. It is assumed that some of the sugar was being used in the formation of tassels, and that such a use would take place more readily in the plants supplied with water than in the plants from which water was withheld.

In the upper dry-leaf cane, the simple sugars decreased in both series, while in the lower dry-leaf cane there was a small increase in both series. At present no explanation of these differences is offered.

*Cane Sugar:*

The results by the three bases of calculation do not always show the same tendencies. The tendencies are the same in the blades and sheaths but different in the stems. The results expressed upon the dry-weight and the residual dry-weight bases agree except in the upper dry-leaf cane, in which no change was indicated in the plants deprived of water, on the dry-weight basis, but on the residual dry-weight basis those plants show a decrease in sucrose. The results on the wet-weight basis show more nearly uniform sucrose content than on the other bases of computation.

Using the residual dry-weight basis of calculation, the following observations may be made:

In the blades, there was an increase in the percentage of sucrose during exposure to light with a greater increase in the plants supplied with water than in the others. Therefore sucrose may be made in the blades of plants which are below the wilting point, but more sucrose is made when the supply of water is adequate. This conclusion agrees with that for simple sugars.

In the sheaths, the sucrose content was a little higher in the plants receiving water than in the others. There was a small increase in both series during the period of illumination. Evidently a little more cane sugar was translocated into the sheaths of the plants receiving water, probably because of the better manufacture of sucrose in the blades of the same plants.

In the green-leaf cane, the plants receiving water decreased in the percentage of sucrose, probably partly due to digestion and utilization in growth and respiration, but also due to translocation as indicated by the considerable increase in the percentage of sucrose in the lower dry-leaf cane of the same plants.

The plants deprived of water surpassed the others in sucrose content in the green-leaf cane. They also contained higher percentages of sucrose in both parts of the dry-leaf cane. Inasmuch as these plants were receiving the same supply of water as the others when the dry-leaf cane was being formed, it is apparent that these differences in sucrose content were not caused by differences in water content during growth. Perhaps the smaller amount of sucrose translocated from the blades into the green-leaf cane of the plants deprived of water, accumulated there due to lack of utilization; whereas in the plants supplied with water so much sucrose was used by further translocation, growth, and respiration that the result was a decrease during the period of illumination. Similarly, in the dry-leaf cane, little sucrose was used in the plants deprived of water, so that the sucrose content remained high during the experiment.

If we explain the lower general level of cane sugar in the dry-leaf cane of the plants supplied with water by assuming greater utilization of the sugar, one may question the possibility of explaining the greater increase in the percentage of cane sugar in the plants receiving water as due to better translocation. Both explanations seem justifiable, however, since the increase due to better translocation occurred during exposure to light, and the decrease due to greater utilization probably occurred during the period of darkness, inasmuch as the sugar content was already low at 8 a. m., the time of the first sampling. In other words, while the plants were in the dark, those receiving water lost more sucrose in their dry-leaf cane than the others, and after they were returned to the light the plants supplied with water were able to translocate more sucrose to their dry-leaf cane because of their greater manufacture of sugar in the blades and perhaps also because of a greater rate of translocation. The plants receiving water are thus assumed to have had a greater rate of metabolism than the plants deprived of water.

#### *Starch:*

The starch content of the blades and sheaths was so low that its presence is questionable. The blades of the plants supplied with water appeared to contain more starch than the blades of the other plants. There is no evidence of the formation of starch in the blades of either series of plants during the period of illumination. Neither is there any evidence in this study of a conversion of starch or of polysaccharides as a whole into sucrose, in the plants below the wilting point, as has been suggested by others workers (3, 4, 12).

In the green-leaf cane, starch decreased during exposure to light, and this decrease was the greater in the plants receiving water, another evidence of their greater metabolic rate.

In the upper dry-leaf cane starch increased during the period of illumination, and in the lower dry-leaf cane there was a small decrease in both series. The plants deprived of water contained more starch than the other plants, in both the upper and lower dry-leaf cane.

#### *Polysaccharides:*

In the blades, there was an increase in the percentage of polysaccharides in the plants supplied with water, but in the other plants the differences were insignificant. Thus photosynthesis and subsequent processes resulted in the formation of polysaccharides only in the plants adequately supplied with water. The blades of the plants deprived of water could conduct enough photosynthesis to form simple sugars and sucrose, but not enough to form starch and other polysaccharides.

That the blades of the plants receiving water produced polysaccharides other than starch is shown by the greater percentages of polysaccharides than of starch and by the increase in polysaccharides during exposure to light, which was unaccompanied by an increase in starch. The nature of these other polysaccharides would form another study.

In the sheaths, there was an accumulation of polysaccharides in both series, with the greater accumulation in the plants supplied with water. That the sheaths of the plants deprived of water accumulated polysaccharides at all is of interest, since the blades of the same plants did not, and is another indication of the lower metabolic rate of those plants, i. e., they may have respired little so there was a surplus of sugar to be stored as polysaccharides in the sheaths, even though none was formed in the blades.

In the green-leaf cane the percentage of polysaccharides increased in both series during exposure to light. The plants deprived of water surpassed the others at both sampling hours, indicating less utilization of carbohydrates by the former.

In the upper and lower dry-leaf cane of both series, there were small changes in the percentage of polysaccharides, which may have no important physiological basis. It is of interest that the plants deprived of water surpassed the others in polysaccharide content, agreeing with the polysaccharide content of the green-leaf cane and with sucrose and starch in the dry-leaf cane.

#### *Total Sugars Plus Polysaccharides:*

The paragraphs dealing with simple sugars, cane sugar, starch, and polysaccharides all indicate the same thing, viz: (1) that although photosynthesis can take place in plants below the wilting point, yet there is more photosynthesis in plants which are adequately supplied with water; that (2) translocation of carbohydrates takes place better in the plants receiving water; but that (3) due to a curtailment in utilization, the storage of carbohydrates is greater in the plants deprived of water.

A summation of the differences in the carbohydrate constituents determined in our study may be obtained by adding the percentages of total sugars and the per-

centages of polysaccharides. The results of this addition are plotted in Fig. 6, using all three of the bases of calculation. This category may be considered to represent the total metabolizable carbohydrate. The sum of the total sugars plus polysaccharides in the blades is an indication of the amount of photosynthate, but it is not a complete figure because some of the photosynthate is translocated immediately and some is used in forming fats, proteins, and other compounds, and some is respired.

In all comparisons, differences on the wet-weight basis are very small, this leveling probably being due to the presence of several factors fluctuating in different directions, some increasing while others decrease. The residual dry-weight basis is considered the most reliable, because it eliminates the fluctuations in water and in carbohydrates, which are the greatest items in fluctuation. In every comparison, the results on the dry-weight basis agree with those on the residual dry-weight basis, the differences being intensified on the latter basis. The following conclusions may be drawn from the results using the dry-weight and residual dry-weight bases:

(1) The synthesis of carbohydrates takes place in the blades of plants which have been kept a few days at a soil moisture content at or below the wilting point. However, the synthesis is greater in the blades of plants adequately supplied with water.

(2) Translocation of carbohydrates into the sheaths takes place in plants at or below the wilting point, but there is more translocation in the plants supplied with water probably because there is a greater source of supply of carbohydrates in the blades of the plants supplied with water.

(3) The carbohydrate content of the stem of the sugar cane plant does not remain equal during the day, there being changes both in the green-leaf cane and in the dry-leaf cane.

(4) In the green-leaf cane, an adequate water supply caused a decrease in carbohydrates, which might be due to greater utilization in growth or better translocation, or both. A similar decrease with similar possible explanations, occurred in the upper part of the dry-leaf cane. None would be used in growth there but it would be used in respiration. Evidence that much of the decrease in carbohydrate in the green-leaf cane and upper dry-leaf cane was due to translocation is indicated by the increase in carbohydrate in the lower dry-leaf cane, which was greater in the plants supplied with water than in those deprived of water. This increase could not be due to photosynthesis. Neither could it be due to condensation alone, as it is an increase in total metabolizable carbohydrate and not in just the higher forms. Therefore this increase represents a supply coming into the lower dry-leaf cane from above.

(5) In the green-leaf cane, the plants deprived of water increased in total metabolizable carbohydrate after they were exposed to the light. The plants from which water was withheld surpassed the others in total metabolizable carbohydrate, not only in the green-leaf cane but also in both parts of the dry-leaf cane. This difference is not due to the growth conditions of the plants, as they all received adequate water while the dry-leaf cane was being formed. The presence of more total metabolizable carbohydrate in the stems of the plants deprived of water than in the others, is a recent difference and is probably due to less utilization during the period of darkness.

### *Which is the Better Plant?*

The plants receiving water were superior in photosynthesis and translocation.

The plants deprived of water were superior in the storage or conservation of carbohydrates in the stem.

If all of the cane sugar present in the stem could be extracted in the mills, then the plants deprived of water are the better from an economic standpoint. Table I, however, shows that the plants receiving water had better juices than the other plants, with one exception. Evidently the sugar present in the plants receiving water was more readily expressed than that in the plants deprived of water.

### *Comparison of the Two Experiments:*

Because the experiment herein reported is the second in a series of studies of water and cane ripening, a comparison of the results of the two tests may be of interest.

The conditions of the experiments differed in several ways. In the first study, the variety D 1135 was used, whereas in the second study the variety H 109 was used. The plants used in the first study were 12 months of age and were not tasseling; those in the second study were 13 months of age and were all tasseling. In the first test the plants had not received uniform treatment prior to the beginning of the experiment, whereas in the second test all of the plants received uniform treatment until the start of the differential treatments. In the first test no analyses could be made of the dry-leaf cane, whereas in the second test analyses of both the upper and lower dry-leaf cane were included.

The results of the moisture determinations were more satisfactory in the first test, inasmuch as in every instance the plants supplied with water contained higher percentages of moisture than the plants deprived of water. In the second test the moisture relationships were irregular, probably because a longer time than usual elapsed between cutting the stalks and weighing the ground samples, due to the unusually large amount of material to be handled.

The plants used in the second test had consistently lower percentages of moisture than the plants of the first test. In the first test, the sheaths of the plants supplied with water had higher percentages of simple sugars than of cane sugar, and the sheaths of the plants deprived of water had lower percentages of simple sugars than of cane sugar. The possibility that the relative amounts of these two forms of sugars are dependent upon the percentage of water has also been indicated in other work (9). In the experiment herein reported the percentages of cane sugar were always higher than the percentages of simple sugars, in every organ, which may be due in part to the consistently lower percentages of moisture in the second experiment.

The trends in some of the carbohydrate constituents differed in the two experiments, in some organs. It would seem, however, that these differences in trends are of minor importance, inasmuch as the data obtained in the two studies lead to similar conclusions. The conclusions of the first experiment are confirmed and amplified by the results of the second experiment.

Both experiments show that photosynthesis may take place at or below the wilting point. In the first test, the blades of the plants deprived of water increased in simple sugars, cane sugar, and polysaccharides, when exposed to sunlight for seven hours after a period of 45 hours in absolute darkness. In the second test, the blades of the plants deprived of water increased in simple sugars and cane sugar but not in polysaccharides.

Both experiments show that photosynthesis takes place better in plants supplied with water than in plants at or below the wilting point. The evidence in the first test consisted in a greater formation of sucrose in the blades of the plants supplied with water than in the others. The evidence in the second test consisted in greater formation of simple sugars and of cane sugar and, in addition, in a formation of polysaccharides in the blades of the plants supplied with water.

In the first test, the explanation of the differences in simple sugars, cane sugar, and polysaccharides in the sheaths and stems could not be definitely determined, because the data were incomplete; it was merely stated that the differences could be explained by assuming either greater utilization of food by the plants supplied with water during seven hours' exposure to light or better translocation in the same plants. The results of the second test, in which more complete data were obtained, indicate that both of these assumptions are required for an adequate explanation. The evidence for this statement is discussed in the sections headed *Cane Sugar* and *Total Sugars plus Polysaccharides*.

#### *Enzyme Activity in Blades:*

Table VII shows that the activity of invertase was greater in series 5 (outdoor controls) than in any of the treated series, whether tested without the addition of buffers or buffered at pH 4.5. Because all of the treated plants were held in the dark for a period of 48 hours, but only half of the plants were deprived of water, it is evident that the decrease in the activity of invertase was a result of the lack of illumination rather than the deprivation of water. Therefore a period of 48 hours in total darkness results in a decrease in the activity of invertase.

In the dark, the activity of invertase was slightly greater in the plants deprived of water than in those supplied with water, whereas in the light the "wet" plants had more active invertase than the "dry" plants. This may indicate that of the plants harvested in the dark, those supplied with water were harmed more in their invertase activity than were those deprived of water; and that of those returned to the light, the plants receiving water increased in their invertase activity while the others did not. Therefore invertase is more responsive to changes in light and darkness when there is plenty of water than when the water supply is deficient. In other words, the activation of invertase in the light and its inactivation in darkness are processes which are favored by a plentiful supply of water. The nature of these changes is not known; they may either be chemical or changes in the colloidal dispersion of the enzyme. The decrease in the dark might be positive adsorption and the increase in the light might be negative adsorption.

Table VIII shows that the most active maltase was found in series 3 (light wet). The activity of maltase in the outdoor control was intermediate, thus differing from invertase, in which the outdoor control was the most active. The 48-hour period of

darkness may have resulted in a slight decrease in maltase in the plants supplied with water, but none was indicated in those deprived of water.

This experiment shows that maltase is less sensitive to darkness than is invertase. Both enzymes when in the dark showed slightly greater activity in the plants deprived of water, and when in the light showed the greater activity in the plants supplied with water.

Table IX shows that of the treated blades, series 3 (light wet) had the most active amylase, which was equal to that of the outdoor control. Withholding water resulted in a decrease in the activity of amylase. Amylase activity in the plants plentifully supplied with water was decreased by darkness; but there is no evidence of a harmful effect of darkness upon the amylase of the plants deprived of water.

Table IX shows greater dextrinase activity in the plants supplied with water than in those deprived of water, both in the dark and the light. There was no evidence of an effect of light upon the activity of dextrinase.

It is interesting that of all the treated blades, series 3 (light wet) had the most active amylase, maltase, and invertase, because as shown in Tables IV and V the same series contained the highest percentages of sucrose and of polysaccharides. Evidently there is a relationship between enzyme activity and chemical composition, but the exact nature of such a relationship is not yet apparent.

When the blades of the plants in the dark and the light (exclusive of the outdoor control) are compared, the following observation may be made. In the light, lack of water was associated with decreased invertase and sucrose, while in the dark, lack of water was associated with increased invertase and sucrose. Therefore it is not lack of water alone which is responsible for the differences, but some factor which develops differently in light and dark as a response to lack of water.

#### *Enzyme Activity in Sheaths:*

Table VII shows the greatest activity of invertase in series 5 only in the unbuffered tests. This may be explained by the hypothesis of Oparin (11), who suggests that McIlvaine's buffers tend to remove enzymes from the adsorbed condition and thus render their activity in different series more nearly equal. The sheaths thus differ from the blades, as in the latter the greatest invertase activity was found in series 5 in both the unbuffered and the buffered tests.

The buffered tests indicate a slight decrease in invertase activity in the dark, and also show that a period of seven hours in the light was sufficient for the invertase to become as active as in the outdoor control. Invertase activity in the sheaths was less severely affected by darkness than that in the blades.

Table VIII shows greater maltase activity in the sheaths of the plants supplied with water than in those deprived of water, in both the dark and the light. Intermediate activity was shown by the outdoor control.

Table IX shows that both amylase and dextrinase were very weak. No differences in activity were found in the different treatments.



### *Enzyme Activity in Green-Leaf Cane:*

Table VII shows nearly equal invertase activity in all series when tested at pH 4.5, in the green-leaf cane. In the unbuffered tests the activity was weaker in series 1 (dark wet) than in the others, for which no explanation is now offered.

Although invertase activity was approximately equal in all the series, in the green-leaf cane, the sucrose content differed as shown in Table IV. Thus, although it is possible that differences in sucrose content may at times cause differences in invertase activity and vice versa, this is not an invariable occurrence. In the present instance the differences in sucrose content were probably due to differences in translocation rather than to immediate differences in enzyme action.

Table VIII shows that the activity of maltase was the greatest in series 4 (light dry), the others being equal. As recorded in Tables IV, V, and VI, series 4 was the highest in simple sugars and polysaccharides but the lowest in starch. Perhaps active maltase aids in speeding up the digestion of starch by hastening the removal of maltose, one of the end products of the digestion of starch. This would lead to a greater percentage of simple sugars, which might then be stored as polysaccharides other than starch.

Table IX shows that neither amylase nor dextrinase activity was detected in the green-leaf cane, but they may have been too weak for detection by our methods.

### *Enzyme Activity in Upper Dry-Leaf Cane:*

Table VII shows that the activity of invertase was very weak in the upper dry-leaf cane. Series 4 (light dry) and 5 (outdoor control) were a little more active than the others, in the unbuffered tests. Differences in maltase activity due to treatment were minor, according to Table VIII. Neither amylase nor dextrinase was detected in the upper dry-leaf cane, according to Table IX. Thus the treatment in this experiment had little effect upon the activity of the enzymes tested in the upper dry-leaf cane.

### *Enzyme Activity in Lower Dry-Leaf Cane:*

The activity of invertase in the lower dry-leaf cane was even weaker than in the upper, as shown in Table VII. So small were the differences that they can hardly be attributed to treatment.

In both the upper and lower dry-leaf cane, the plants deprived of water had the more sucrose, polysaccharides, and starch, as shown in Tables IV, V, and VI. Neither polysaccharides as a whole nor starch in particular can be translocated. Therefore they must be manufactured in place. Yet no amylase or dextrinase was detected according to Table IX. It is not possible to say that these carbohydrates were stored when the stem was young and contained more active enzymes, for then how would we explain the differences in carbohydrate content? The differences are recent and they occurred in place. Therefore either greater differences in enzyme action occurred in the living plant than were determined by our methods, or else starch may be synthesized in the presence of weak amylase and dextrinase when the conditions favor condensation (or union due to withdrawal of water).

### *The Effect of Water Upon Enzymes:*

The effect of water upon the activity of the enzymes studied in this investigation is summarized in Table X, which shows that in the dark, water decreased the action of invertase and maltase but increased that of amylase and dextrinase in the blades. Water increased the activity of maltase in the sheaths. The enzymes in the other organs seemed not to be affected by the water supply, in the dark.

TABLE X  
Effect of water upon enzymes.\*

Organ	Invertase in the dark	Maltase	Amylase	Dextrinase
Blades .....	—	—	+	+
Sheaths .....	0	+	0	0
Green-leaf cane .....	0	0	0	0
Upper dry-leaf cane.....	0	0	0	0
Lower dry-leaf cane.....	0	0	0	0
	in the light			
Blades .....	+	+	+	+
Sheaths .....	0	0	0	0
Green-leaf cane .....	0	—	0	0
Upper dry-leaf cane.....	0	—	0	0
Lower dry-leaf cane.....	0	0	0	0

\* + = water increases action; — = water decreases action; 0 = no effect of water.  
(For invertase and maltase, differences less than 1 cc. N/20 KMnO<sub>4</sub>.)

In the light, water increased the activity of the four enzymes studied in the blades. No other effect was shown except that water decreased the action of maltase in the green-leaf cane and in the upper dry-leaf cane.

Therefore the enzymes in the blades are more sensitive to differences in water supply than are those in the other organs of the plant. Further discussion of the effect of water upon enzyme activity and chemical composition will be postponed until the experiment has been repeated.

### SUMMARY

1. Certain aspects of the effect of water upon photosynthesis, translocation, storage of sugar, and enzyme activity were studied in sugar cane of the variety H 109.
2. Photosynthesis can take place in plants below the wilting point, as shown by increases in simple sugars and cane sugar in the blades of plants exposed to sunlight for seven hours after a period of 48 hours in absolute darkness.
3. Although photosynthesis can take place in plants below the wilting point, yet there is more photosynthesis in plants which are adequately supplied with water, as shown by their greater increases in simple sugars and cane sugar, and in addition by an increase in polysaccharides, under the conditions described in the preceding paragraph.
4. The translocation of carbohydrates takes place better in plants receiving an adequate supply of water than in plants below the wilting point.

5. Due to a curtailment in utilization, the storage of carbohydrates is greater in the plants deprived of water than in those supplied with water.

6. Although the plants deprived of water had the higher percentages of cane sugar in the tissue of the stem, the plants receiving water yielded in general the better juices, indicating that the sugar present in the plants receiving water was more readily expressed than that in the plants deprived of water.

7. A period of 48 hours of total darkness results in a decrease in the activity of invertase in the leaf-blades of the sugar cane plant.

8. The invertase in the blades is more responsive to changes in light and darkness when there is plenty of water than when the water supply is deficient.

9. The maltase in the blades is less sensitive to darkness than is invertase.

10. Withholding water resulted in an increase in the activity of invertase in the blades in the dark, but a decrease in the light. The activity of invertase in the other organs of the sugar cane plant seemed not to be affected by the water supply.

11. Withholding water resulted in a decrease in the activity of amylase and dextrinase in the blades.

12. The series of treated plants which had the most active invertase, amylase, and maltase in the blades was the series which had the highest percentages of sucrose and of polysaccharides in the blades.

13. The enzymes of the blades of the sugar cane plant (i. e., invertase, amylase, dextrinase, and maltase, the only ones studied) are sensitive to differences in moisture and light; the enzymes in the sheaths are less sensitive than those in the blades; the only enzyme in the green-leaf cane apparently affected by treatment was maltase; and in the dry-leaf cane any differences due to treatment were so small as to be insignificant.

#### *Acknowledgments:*

This experiment was undertaken in collaboration with Dr. Mangelsdorf and Mr. Wadsworth and the author takes pleasure in expressing her appreciation of their cooperation. Credit is due the Sugar Technology Department for conducting the juice analyses, and to Mr. Sa Ning for the darkening and the heating of the assembly room. Grateful appreciation is expressed in particular to Ada Forbes for her constant assistance during the experiment, and to Dr. Lyon for his valuable advice and helpful suggestions.

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## Sugar Prices

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96° CENTRIFUGALS FOR THE PERIOD  
JULY 7, 1936, TO SEPTEMBER 3, 1936.

Date	Per Pound	Per Ton	Remarks
July 7, 1936.....	3.71¢	\$74.20	Cubas.
“ 8.....	3.70	74.00	Puerto Ricos.
“ 28.....	3.65	73.00	Puerto Ricos.
Aug. 6.....	3.67	73.40	Philippines.
“ 13.....	3.70	74.00	Puerto Ricos.
Sept. 3.....	3.65	73.00	Virgin Islands.

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# INDEX TO VOLUME XL

(An asterisk preceding a page number indicates that the subject is illustrated.)

## A

- Acid, phosphoric—  
 available in sugar cane soils. \* 3  
 in boiler water, by R.C.M. 265  
 in filter cake, by R.C.M. 265  
 in mill ash, by R.C.M. 278  
*Anagrus frequens* Perkins. 57  
 Annual synopsis of mill data—1935 (see Circular No. 66)  
*Aphytus terryi*, in Guam. 309  
 Ash, mill, potash in, by R.C.M. \* 232, 278  
 Assassin bug, *Zelus renardii* Kolenati. \* 57  
 Australia, the giant Macadamia nut. \* 303

## B

- Bagasse—  
 burning quality of P.O.J. 2878. 118  
 digestibility by animals. 123  
 Baits, poison, for rat control. 157  
 Balanced block arrangement of treatments. \* 113  
 Banana, in Guam, pests and parasites. 310  
 Base exchange, a discussion of. 319  
 Beans, in Guam, pests and parasites. 312  
 Biological control of sugar cane leafhopper in Hawaii. \* 57  
 Boiler(s)—  
 factory, primary and total combustion volume in. 183  
 water, determination of phosphates in, by R.C.M. \* 229  
 water, estimation of phosphoric acid in, by R.C.M. 265  
 Borden, R. J.—  
 cane growth studies. \* 143  
 diagnosing the status of available phosphoric acid and potash in sugar cane soils. \* 3  
 some interesting cane-tonnage, purity, soil-analyses, juice-analyses relationships. \* 11  
 studies in experimental technique. \* 113  
 Border effect. 113  
 Boron—  
 in nutrient solutions. \* 137  
 see fertilizers.  
 Brix, observations at Pioneer Mill Company, Ltd. \* 21  
 By-products, mill (ash, filter cake and molasses)—  
 phosphate in, by R.C.M. \* 229  
 phosphoric acid in, by R.C.M. 265  
 potash in, by R.C.M. \* 232, \* 233, 273, 278  
 total calcium in, by R.C.M. \* 234, 285  
 total nitrogen in, by R.C.M. \* 221, 246, 258

## C

- Cabbage, in Guam, pests and parasites. 312  
 Calcium—  
 as discussed at Third International Congress of Soil Science. 130  
 in nutrient solutions. \* 137  
 readily soluble in soil, by R.C.M. \* 233, 281  
 see fertilizers.  
 total, in filter cake, by R.C.M. \* 234, 285  
 Cane—  
 annual synopsis of mill data—1935 (see Circular No. 66).  
 diseases, see diseases.  
 fluctuations of sugars in, during the day and night. \* 329  
 growth studies. \* 143  
 in Guam, pests and parasites. 307  
 in nutrient solutions. \* 137  
 juices, see juices.  
 nitrogen content by R.C.M. \* 221, 254  
 pests, see pests.  
 P.O.J. 2878 in the factory. 117  
 rind hardness. \* 815  
 ripening, notes on. \* 855

- some aspects of the internal water economy of. \* 21  
 tasseled versus untasseled in rind hardness. \* 815  
 the effect of nitrogen on yield and juice quality. \* 35  
 tonnage, purity, soil, juice relationships. \* 11  
 varieties, see varieties.  
 yields in relation to phosphate and potash in juice and soil. \* 3, \* 11  
 Carpenter, C. W., notes on *Pythium* root rot—VIII. \* 137  
 Cattle, utilization of molasses in fattening. 121  
 Caum, E. L., yams for Hawaiian gardens. \* 171  
*Ceromazia sphenophori*, in Guam. 309  
*Chelinoches morio* (Fab.). \* 57  
*Chrysopa microphya* McLachlan. \* 57  
 Climate, dominating effect on cane growth. \* 143  
 Coconut, in Guam, pests and parasites. 307  
*Coelophora inaequalis* (Fab.). \* 57  
 Color standards, used in R.C.M. work. \* 189, 239  
 Colorimetric determination of soil reaction (pH). \* 234, 286  
 Combustion, volume, primary and total in factory boilers. 183  
*Conocephalus saltator* (Saus.). \* 57  
 Corn, in Guam, pests and parasites. 308  
 Cornelison, A. H., the effect of nitrogen on cane yield and juice quality. \* 35  
*Cyrtorhinus mundulus* (Breddin). \* 57

## D

- Das, U. K.—  
 a suggested scheme of irrigation control using the day-degree system. \* 109  
 the day-degree in Mauritius. \* 103  
 the effect of nitrogen on cane yield and juice quality. \* 35  
 Davis, L. E., soil reaction and total acidity. 319  
 Day-degree—  
 in irrigation control. \* 109  
 in Mauritius. \* 103  
 Diagnosing the status of available phosphoric acid and potash in sugar cane soils. \* 3  
 Diseases—  
 cane, *Pythium* root rot. \* 137  
 cane, rust, in Guam. 310  
 leafhoppers, fungus, *Cordyceps*. 57  
 leafhoppers, fungus, *Entomophthora* sp. 57  
 leafhoppers, fungus, *Sporotrichum* sp. 57  
 mealybugs, fungus, in Guam. 309

## E

- Earwigs—  
*Chelinoches morio* (Fab.). \* 57  
*Euborellia annulipes* (Lucas). \* 57  
*Eckthrodelphez fairchildii* Perkins. \* 57  
 Eckart, Thomas G., rat control investigations at The Lihue Plantation Company, Ltd. \* 157  
 Electrical conductivity of cane juice. \* 52  
 Entomology department, organization of, at Experiment Station. 65  
*Euborellia annulipes* (Lucas). \* 57  
 Experiments—  
 control investigations on rats. 157  
 feeding of beef cattle. 124  
 fluctuations of sugars in cane during the day and night. \* 329  
 further notes on water and cane ripening. \* 355  
 studies in technique. \* 113  
 studies on effects of climate. \* 143

## F

- Fertilizer(s)—  
 boron. \* 137  
 calcium. 180, \* 137, \* 189  
 filter cake. \* 189

iron	187
magnesium	187
manganese	184, 187
molasses	189
nitrogen	* 85, * 137, 189
phosphate	* 3, * 11, * 134, 189
phosphoric acid	3
phosphorus	* 137
potash	* 8, * 11, * 189
potassium	* 137
Filter cake—	
phosphate in, by R.C.M.	* 229
phosphoric acid in, by R.C.M.	265
see fertilizers.	
total calcium in, by R.C.M.	* 234, 285
total nitrogen in, by R.C.M.	* 221, 246

Flies—	
<i>Anomalochrysa deceptor</i> Perk.	57
<i>Anomalochrysa gayi</i> Perk.	57
<i>Anomalochrysa proteus</i> Perk.	57
<i>Anomalochrysa raphidiotides</i> Perk.	57
<i>Chrysopa microphya</i> McLachlan.	* 57
house, in Guam.	314
<i>Pipunculus hawaiiensis</i> Perk.	* 57
<i>Pipunculus fuvator</i> Perk.	* 57
<i>Pipunculus terryi</i> Perk.	* 57
Forest insects, in Guam.	312
Fungus disease—	
of leafhoppers	57
of mealybugs, in Guam.	309

## G

Grasshoppers—	
<i>Conocephalus saltator</i> (Sayss.).	* 57
in Guam	310
Grove Farm Company, Ltd., investigates	
soil testing kits.	195
Growth—	
length of H 109.	* 37
measurements, detailed	* 28
studies, cane	* 143
Guam, a preliminary report on an entomological survey	307.

## H

H 109 cane—	
cane-tonnage, purity, soil-analyses,	
juice-analyses relationships	* 11
effect of climate on.	* 143
effect of nitrogen on yield and juice	
quality	* 35
effect of water on ripening.	* 355
in nutrient solutions.	* 137
length growth	* 37
see varieties.	
water economy	* 21
Hance, Francis E.—	
soil and plant material analyses by	
rapid chemical methods.	* 189
soil reaction and total acidity.	319
the Third International Congress of	
Soil Science	127
<i>Haplogonotopus vittensis</i> Perkins.	57
Hart, Constance E.—	
further notes on water and cane ripening	* 355
the fluctuations of sugars in the leaf	
sheaths of the sugar cane plant	
during the day and the night.	* 329

## I

Insects—	
see parasites,	
see pests.	
see predators.	
Instrument, for measuring comparative	
hardness of canes.	* 315
International Congress of Soil Science, Third	127
Iron—	
in nutrient solutions.	* 137
see fertilizers.	
Irrigation—	
use of day-degree in.	* 109
water, in connection with cane ripening	
ing	* 355
water, nitrogen in, by R.C.M.	* 223, 249, 250
water, potash in, by R.C.M.	* 232, 274
water, pump, potash in, by R.C.M.	276

Juices, cane—	
analyses, cane tonnage, soil, relation-	
ships	* 11
annual synopsis of mill data—1935	
(see Circular No. 66)	
electrical conductivity of.	* 52
of POJ 2878, in the factory.	117
phosphate in, by R.C.M.	* 227, 263
potash in, by R.C.M.	* 231, 271
studies of phosphoric acid and potash	
in	* 3, * 11
the effect of nitrogen on quality.	* 35
total nitrogen in, by R.C.M.	* 221, 251, 253

## K

Koebele, Albert, work on leafhopper control.	57
--	----

## L

Laboratories, plantation, views of R.C.M.	* 289
Lady beetles, <i>Coelophora inaequalis</i> (Fab.).	* 57
Lamb, A. R., utilization of molasses.	121
Leafhopper—	
biological control in Hawaii.	* 57
early history in Hawaii.	58
fungus diseases of.	98
Lihue Plantation Company, Ltd., rat control	
investigations	157

## M

Macadamia nut, giant.	* 303
Magnesium—	
in nutrient solutions.	* 137
see fertilizers.	
Manganese—	
as discussed at Third International Con-	
gress of Soil Science.	134
in nutrient solutions.	* 137
see fertilizers.	
Mango, in Guam, pests and parasites.	311
Martin, J. P., yams for Hawaiian gardens.	* 171
Mauritius, use of day-degree in.	* 103
McCleery, W. L.—	
annual synopsis of mill data—1935 (see	
Circular No. 66)	
mill roller openings.	183
POJ 2878 cane in the factory.	117
primary and total combustion volume	
in factory boilers.	183
Mill—	
ash, determination of phosphate in, by	
R.C.M.	* 229
ash, potash in, by R.C.M.	* 232, 278
by-products, phosphate in, by R.C.M.	* 229
by-products, phosphoric acid in, by R.	
C.M.	265
by-products, potash in, by R.C.M.	* 233, 273
by-products, total calcium in, by R.C.M.	* 234, 285
by-products, total nitrogen in, by R.	
C.M.	* 221, 246, 258
data, annual synopsis of—1935 (see	
Circular No. 66)	
roller openings	183
waters, nitrogen in, by R.C.M.	* 223, 249, 250
Moisture content of cane.	* 21
Molasses	
energy in	122
potash in, by R.C.M.	* 233, 273
see fertilizers.	
total nitrogen in, by R.C.M.	* 221, 258
utilization in fattening cattle.	121
Mosquitoes, in Guam.	313

## N

<i>Nesomimeea hawaiiensis</i> Perk.	* 57
Nitrogen—	
ammoniacal, in soils, by R.C.M.	* 219, 245
available, in soils, by R.C.M.	* 219, 243
color standards (R.C.M.).	* 189, 242
in nutrient solutions.	* 137
in irrigation and mill waters, by R.C.M.	* 223, 249, 250
nitrate, in soils, by R.C.M.	* 221, 245
see fertilizers.	

studies, by R.O.M.	254
the effect on cane yields and juice quality	* 35
total, in cane juice, by R.C.M.	* 221, 251, 253
total, in mill by-products, by R.C.M.	* 221, 246, 258
total, in plant material, by R.C.M.	* 221, 254
total, in soil, by R.C.M.	* 221, 248
Nut, giant Macadamia	* 303

## O

<i>Oechalia grisea</i> (Burm.)	* 57
<i>Ootetrastichus</i> —	
<i>beatus</i> Perkins	* 57
<i>formosanus</i> Timberlake	* 57
Orange, in Guam, pests and parasites	311

## P

<i>Paranagrus</i> —	
a species in Guam	309
<i>optabilis</i> Perkins	* 57
<i>perforator</i> Perkins	* 57
Parasites—	
<i>Anagrus frequens</i> Perkins	57
<i>Aphyus terryi</i> , in Guam	309
<i>Ceromasia sphenophori</i> , in Guam	309
<i>Echthrodelpfax fairchildii</i> Perkins	* 57
<i>Haplogonatopus vitiensis</i> Perkins	57
<i>Ootetrastichus beatus</i> Perkins	* 57
<i>Ootetrastichus formosanus</i> Timberlake	* 57
<i>Paranagrus</i> , a species in Guam	309
<i>Paranagrus optabilis</i> Perkins	* 57
<i>Paranagrus perforator</i> Perkins	* 57
<i>Pipunculus hawaiiensis</i> Perk.	* 57
<i>Pipunculus juvator</i> Perk.	* 57
<i>Pipunculus terryi</i> Perk.	* 57
<i>Pseudogonatopus hospes</i> Perkins	* 57
Pemberton, O. E.—	
a giant Macadamia nut	* 303
comparative hardness of tasseled versus untasseled canes	* 315
Perkins, R. C. L., work on leafhopper control	57
<i>Perkinsiella</i> —	
<i>saccharicida</i> Kirkaldy, the sugar cane leafhopper	* 57
<i>thompsoni</i> , in Guam	309
Pests of sugar cane—	
cane borer, <i>Rhabdocnemis obscura</i> , in Guam	309
cane borer, <i>Rhabdocnemis obscura</i> , in tasseled versus untasseled cane	* 315
leafhoppers, biological control	* 57
<i>Perkinsiella saccharicida</i>	* 57
<i>Perkinsiella thompsoni</i> , in Guam	309
<i>Pseudococcus boninensis</i> , the gray mealybug, in Guam	309
<i>Pseudococcus brevipes</i> , the pineapple mealybug, in Guam	310
rats, control investigations	157
<i>Trionymus sacchari</i> , the pink mealybug, in Guam	309
pH, (soil reaction)	128, * 234, 286, 319
Phosphate—	
as discussed at Third International Congress of Soil Science	134
available, in sugar cane soils	* 3
color standards (R.C.M.)	* 189, 240, 241
fixation, estimation of, by R.C.M.	* 225, 261
fixation solutions	238
free or soluble in boiler water, by R. C.M.	* 229
in cane juice, by R.C.M.	* 227, 263
in filter cake, by R.C.M.	* 229
in juice and soil	* 11
in mill ash, by R.C.M.	* 229
readily soluble in soil, by R.C.M.	* 224, 259
see fertilizers.	
Phosphoric acid—	
available in sugar cane soils	* 3
in boiler water, by R.C.M.	265
in filter cake, by R.C.M.	265
in mill ash, by R.C.M.	278
see fertilizers.	
Phosphorus—	
in nutrient solutions	* 137
see fertilizers.	
<i>Physopleurella mundulus</i> (White)	* 57
Pineapple, in Guam, pests and parasites	311
Pioneer Mill Company, Ltd.	* 3, * 11, * 21

<i>Pipunculus</i> —	
<i>hawaiiensis</i> Perk.	* 57
<i>juvator</i> Perk.	* 57
<i>terryi</i> Perk.	* 57
Plant material—	
analyses, by R.O.M.	* 189
total nitrogen in, by R.C.M.	* 221, 254
Poison, bait for rat control	157
POJ 2878 cane—	
cane-tonnage, purity, soil-analyses, juice-analyses relationships	* 21
effect of climate on	* 143
in the factory	117
see varieties.	
Potash—	
available, in sugar cane soils	* 3
in cane juice, by R.C.M.	* 231, 271
in irrigation water, by R.C.M.	* 232, 274
in juice and soil	* 11
in mill ash, by R.C.M.	* 232, 278
in molasses, by R.C.M.	* 233, 273
in pump water, by R.C.M.	276
in soil, determination of readily soluble by R.C.M.	* 280, 267
see fertilizers.	
Potassium—	
in nutrient solutions	* 137
see fertilizers.	
Predators—	
<i>Anomalochrysa deceptor</i> Perk.	57
<i>Anomalochrysa gayi</i> Perk.	57
<i>Anomalochrysa proteus</i> Perk.	57
<i>Anomalochrysa raphidioides</i> Perk.	57
<i>Cheliasches morio</i> (Fab.)	* 57
<i>Chrysopa microphya</i> McLachlan	* 57
<i>Coelophora inaequalis</i> (Fab.)	* 57
<i>Conocephalus saltator</i> (Sauss.)	* 57
<i>Cyrtorhinus mundulus</i> (Breddin)	* 57
<i>Euborellia annulipes</i> (Lucas)	* 57
<i>Oechalia grisea</i> (Burm.)	* 57
<i>Physopleurella mundulus</i> (White)	* 57
<i>Reduviolus blackburni</i> (White)	* 57
<i>Reduviolus capsiformis</i> (Germar)	* 57
spiders	* 57
<i>Triphleps persequens</i> White	* 57
<i>Zelus renardii</i> Kolenati	* 57
Prices of Sugar—	
Sept. 16, 1935—Dec. 7, 1935	104
Dec. 30, 1935—March 11, 1936	185
March 23, 1936—June 3, 1936	300
July 7, 1936—Sept. 3, 1936	382
<i>Pseudococcus</i> —	
<i>boninensis</i> , the gray mealybug in Guam	309
<i>brevipes</i> , the pineapple mealybug in Guam	310
<i>Pseudogonatopus hospes</i> Perkins	* 57
<i>Pythium</i> , root rot, notes on	* 137

## Q

Quality ratio, annual synopsis of mill data 1935
(see Circular No. 66)

## R

Rapid chemical methods—	
color standards	* 189, 239
detailed procedures	243
history and introduction in Hawaii	* 189
nitrogen studies	254
soil and plant material analyses by	* 189
Rats, control investigations at The Lihue Plantation Company, Ltd.	157
Reagents, used in R.C.M. work	* 189, 235
<i>Reduviolus</i> —	
<i>blackburni</i> (White)	* 57
<i>capsiformis</i> (Germar)	57
<i>Rhabdocnemis obscura</i> —	
in Guam	309
in tasseled versus untasseled cane	* 315
Rice, in Guam, pests and parasites	309
Rust, cane, a disease in Guam	310

## S

Seedlings, see varieties.	
Soil (s)—	
ammoniacal nitrogen in, by R.C.M.	* 219, 245
analyses by rapid chemical methods...	* 189



analyses, cane tonnage, juice, relation-	
ships . . . . .	* 11
available nitrogen in, by R.C.M. . . . .	* 219, 248
diagnosing the status of available phos-	
phoric acid and potash in . . . . .	* 3
Makiki and Manoa, in experiment . . . .	* 148
nitrate nitrogen in, by R.C.M. . . . .	* 221, 245
phosphate fixation (R.C.M.) . . . . .	* 225, 261
reaction (pH) . . . . .	* 128, * 284, 286, 819
readily soluble calcium in, by R.C.M. . .	* 238, 281
readily soluble phosphate in, by R.C.M. .	* 224, 259
readily soluble potash in, by R.C.M. . . .	* 280, 267
Science, Third International Congress	
of . . . . .	127
survey . . . . .	128
total nitrogen in, by R.C.M. . . . .	* 221, 248
Spiders—	
<i>Adrasidea nebulosa</i> Simon . . . . .	57
<i>Bavia aericeps</i> (Sim.) . . . . .	57
Enemies of . . . . .	57
<i>Hasarius adansoni</i> (Aud.) . . . . .	57
<i>Heteropoda regia</i> (Fab.) . . . . .	57
<i>Molika microtholmus</i> (Koch.) . . . . .	57
<i>Pagiopalus atomarius</i> Simon . . . . .	* 57
<i>Plexippus paykulli</i> (Aud.) . . . . .	57
<i>Tetragnatha mandibulata</i> Walck. . . . .	* 57
Striped Tip cane—	
effect of climate on . . . . .	* 143
see varieties. . . . .	
Sugar cane—	
diseases, see diseases. . . . .	
in Guam . . . . .	309
irrigation, see irrigation. . . . .	
juices, see juices. . . . .	
pests, see pests. . . . .	
prices . . . . .	104, 185, 300, 382
rind hardness . . . . .	* 315
Sugar yield, annual synopsis of mill data—	
1935 (see Circular No. 66) . . . . .	
Sugars, fluctuations of, in leaf sheaths of	
cane plant during the day and	
night . . . . .	* 329
Swezey, O. H.—	
a preliminary report on an entomologi-	
cal survey of Guam . . . . .	307
biological control of the sugar cane leaf-	
hopper in Hawaii . . . . .	* 57

## T

Taro, in Guam, pests and parasites . . . .	310
Temperature, in relation to the internal wa-	
ter economy of the cane plant . . . . .	* 21

Third International Congress of Soil Science	127
Tobacco, in Guam, pests and parasites . .	310
<i>Trionymus sacchari</i> , the pink-mealybug in	
Guam . . . . .	309
<i>Triphleps persequens</i> White . . . . .	* 57

## V

Varieties of sugar cane—	
annual synopsis of mill data—1935	
(see Circular No. 66) . . . . .	
H 109 . . . . .	* 11, * 21, * 35, * 37, 117, * 137, * 143, * 355
POJ 2878 . . . . .	* 21, 117, * 143
rind hardness . . . . .	* 315
striped tip . . . . .	* 143
Yellow Caledonia . . . . .	* 21

## W

Wadsworth, H. A., some aspects of the in-	
ternal water economy of the sugar	
cane plant . . . . .	* 21
Waimanalo Sugar Company, POJ 2878 cane	
in the factory . . . . .	117
Wasps—	
as enemies of spiders . . . . .	57
<i>Nesomimesa hawaiiensis</i> Perk. . . . .	* 57
Water—	
boiler, free or soluble phosphate in,	
by R.C.M. . . . .	* 229
boiler, phosphoric acid in, by R.C.M. . .	265
content of cane plant . . . . .	* 44
economy in cane plant . . . . .	* 21
mill, nitrogen in, by R.C.M. . . . .	* 223, 249, 250
notes on, in connection with ripening .	* 355
pump, potash in, by R.C.M. . . . .	276
see irrigation. . . . .	
use of day-degree in irrigation . . . . .	* 109

## Y

Yams, for Hawaiian gardens . . . . .	* 171
Yellow Caledonia—	
cane-tonnage, purity, soil-analyses,	
juice-analyses relationships . . . . .	* 21
see varieties. . . . .	

## Z

<i>Zelus renardii</i> Kolenati . . . . .	* 57
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